PATENT IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application No.:	10/759,746
Applicant:	Fernandez-Salas, E., *et al.*
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Customer No.:	51957
Patent Title:	Methods of Identifying Compounds that Alter Toxin Persistence and/or Protease Activity

FILED ELECTRONICALLY

APPEAL BRIEF

November 9, 2007

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir,

This 61 page communication is in response to a Notice of Non-Compliant Appeal Brief mailed on October 10, 2007.

Respectfully submitted,

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I. REAL PARTY IN INTEREST

The real party in interest is Allergan, Inc., the assignee of record, having a principal place of business at 2525 Dupont Drive, Irvine, California, 92612.

II. RELATED APPEALS AND INTERFERENCES

Co-pending U.S. Patent Application No.: 10/163,106 is currently under appeal with the Board of Patent Appeals and Interferences. This co-pending application is a continuation in part of the present patent application.

III. STATUS OF CLAIMS

The status of the claims is as follows: Claims 1, 3-20, 22, 45-47, 56 and 57 are pending and stand rejected. Claims 2, 21, 23-44 and 48-55 were canceled. The Applicants appeal all of the rejected claims.

IV. STATUS OF AMENDMENTS

The Applicants filed an Applicants' Reply on June 29, 2007 indicating that the claim amendments satisfied the written description requirement pursuant to 35 U.S.C. § 112, ¶ 1 because the specification amendment pertaining to the incorporation by reference of U.S. Patent Application 10/757,077 was appropriate pursuant to According to *MPEP* § 608.01(p) and request withdrawal of the objection to this amendment because the amended specification does not introduce new matter into the disclosure. The Examiner issued an Advisory Action of July 23, 2007 indicating that the objection to the amendment was maintained because 1) the specification fails to point to specific portions of the referenced document where the subject matter being incorporated may be found; 2) the specification fails to indicate the relationship between the instant application and the cited application under *MPEP* § 201.11(II).

V. SUMMARY OF CLAIMED SUBJECT MATTER

Botulinum toxin type A (BoNT/A) is an approximately 150 kDa neurotoxin of 1296 amino acids in length. *See, e.g.*, D. Borden Lacy and Raymond C. Stevens, *Sequence Homology and Structural Analysis of the Clostridial Neurotoxins*, 291(5) J. Mol. Biol. 1091-11-4 (1999) at 1092, Figure. 1; and 1094, Figure. 3. Each mature BoNT/A comprises three functionally distinct domains: 1) an enzymatic domain located in the light chain having endopeptidase activity that specifically targets membrane-bound SNAP-25; 2) a translocation domain contained within the amino-terminal half of the heavy chain (H_N) that facilitates release of the light chain from intracellular vesicles into the cytoplasm of the target cell; and 3) a binding domain found within the carboxyl-terminal half of the heavy chain (H_C) that determines the binding activity and binding specificity of the toxin to the receptor complex located at the surface of the target cell. *See, e.g.*, D. Borden Lacy et al., *Crystal Structure of Botulinum Neurotoxin Type A and Implications for Toxicity*, 5(10) Nature Struct. Biol. 898-902 (1998) at 899-902.

The overall cellular intoxication mechanism whereby BoNT/A enter a neuron and inhibit neurotransmitter release can be described as comprising four steps: 1) receptor binding, 2) toxin internalization, 3) light chain translocation, and 3) intracellular target action. *See,* Present specification at ¶¶ 13-16; Cesare Montecucco and Giampierto Schiavo, *Structure and Function of Tetanus and Botulinum Neurotoxins*, 28(4) Q. Rev. Biophys. 423-472 (1995) at 434-445; Lacy, 5(10) Nature Struct. Biol. at 899-902; Lacy, 291(5) J. Mol. Biol. at 1091, col. 2, ¶ 2, line 1 through p. 1092, col. 1, ¶ 1, line 30; and Giampierto. Schiavo et al., *Neurotoxins Affecting Neuroexocytosis*, 80(2) Physiol Rev 717-766 (2000) at 725-731. The process is initiated when the binding domain of BoNT/A binds to a BoNT/A-specific receptor complex located on the plasma membrane surface of a target cell (receptor binding step). Once bound, the toxin/receptor complexes are internalized by endocytosis and the internalized vesicles are sorted to specific intracellular routes (toxin internalization). While in the vesicle, the light chain is separated from the heavy chain, and through a series of events involving the translocation domain, the separated BoNT/A light chain is released from the intracellular vesicle into the cytosol (light chain translocation step). Once in the cytosol, the light chain localizes to the plasma membrane where it can specifically cleaves its substrate, SNAP-25 (intracellular target action step).

The presently claimed method is directed towards a method of identifying a compound that either reduces or increases a biological persistence of a BoNT/A by using a test localization assay. *See*, *e.g.*, Present specification at ¶¶ 22 and 56. This method takes advantage of the fact that membrane localization of the BoNT/A light chain is a key factor in controlling the length of time a BoNT/A light chain can exert its effects on a cell, *i.e.*, biological persistence. *See, e.g.*, *Id.* at ¶ 17. The presently claimed screen method identifies compounds that can affect biological persistence by determining with that compound alters the amount of time a BoNT/A light chain remains associated with the plasma membrane. *See, e.g.*, *Id.* at ¶¶ 22, 36, 46 and 56. A compound that reduces biological persistence of BoNT/A will be one that reduces the length of time the BoNT/A light chain remains associated with the plasma membrane relative to a control. *See, e.g.*, *Id.* at ¶¶ 23, 52, and Examples 5 & 6. Conversely, a compound that increases biological persistence of BoNT/A will be one that increases the length of time the BoNT/A light chain remains associated with the plasma membrane relative to a control. *See, e.g.*, *Id.* at ¶¶ 24, 55 and 56.

The method steps comprise a) contacting a cell that has a BoNT/A comprises a BoNT/A light chain with a test compound, wherein the BoNT/A light chain displays an intracellular localization pattern at the plasma membrane; b) observing the membrane localization pattern of the BoNT/A light chain following contact of the cell with the test compound; and c) comparing the observed BoNT/A light chain membrane localization pattern to a membrane localization pattern of a BoNT/A light chain in a cell in an absence of the test compound; wherein a reduced membrane localization pattern of the BoNT/A light chain over time in the cell contacted with the test compound as compared to the membrane localization pattern of the BoNT/A light chain over time in the cell in the absence of the test compound is indicative of a test compound that reduces the biological persistence of a BoNT/A; and wherein an increased membrane localization pattern of the BoNT/A light chain over time in the cell contacted with the test compound as compared to the membrane localization pattern of the BoNT/A light chain over time in the cell in the absence of the test compound is indicative of a test compound that increases the biological persistence of a BoNT/A. See, *infra*, p. 45, Claim 1.

Independent Claim 1 is directed to a method of identifying a compound that either reduces or increases a biological persistence of a BoNT/A, the method comprising a test localization assay (¶¶ 22; 56; 115; and Original Claim 1) comprising the steps of a) contacting a cell that comprises a BoNT/A light chain with a test compound, wherein the BoNT/A light chain displays an intracellular localization pattern at the plasma membrane (¶¶ 36; 56; 116; and Original Claim 1); b) observing the membrane localization pattern of the BoNT/A light chain following contact of the cell with the test compound (¶¶ 56; 117; and Original Claim 1); and c) comparing the observed BoNT/A light chain membrane localization pattern to a membrane localization pattern of a BoNT/A light chain in a cell in an absence of the test compound (¶¶ 56; 118; and Original Claim 1); wherein a reduced membrane localization pattern of the BoNT/A light chain over time in the cell contacted with the test compound as compared to the membrane localization pattern of the BoNT/A light chain over time in the cell in the absence of the test compound is indicative of a test compound that reduces the biological persistence of a BoNT/A (¶¶ 23; 52; Examples 5 & 6; and Original Claims 3 & 4); and wherein an increased membrane localization pattern of the BoNT/A light chain over time in the cell contacted with the test compound as compared to the membrane localization pattern of the BoNT/A light chain over time in the cell in the absence of the test compound is indicative of a test compound that increases the biological persistence of a BoNT/A (¶¶ 24; 55; 56; and Original Claim 1).

Independent Claim 45 is directed to a method of identifying a compound that either reduces or increases a biological persistence of a BoNT/A, the method comprising the steps of a) contacting a cell that comprises a BoNT/A light chain with a test compound, wherein the BoNT/A light chain displays an intracellular localization pattern at the plasma membrane (¶¶ 36; 56; 116; Original Claims 33 and 45); and b) determining whether the membrane localization pattern of the BoNT/A light chain is reduced or increased in the cell contacted with the test compound as compared to the membrane localization pattern of the BoNT/A light chain in a cell in the absence of the test compound (Original Claims 33 and 45), wherein a reduced membrane localization pattern of the BoNT/A light chain over time in the cell contacted with the test compound as compared to the membrane localization pattern of the BoNT/A light chain over time in the cell in the absence of the test compound is indicative of a test compound that reduces the biological persistence of a BoNT/A (¶¶ 23, 52, Examples 5 & 6; and Original Claim 45); and wherein an increased membrane localization pattern of the BoNT/A light chain over

time in the cell contacted with the test compound as compared to the membrane localization pattern of the BoNT/A light chain over time in the cell in the absence of the test compound is indicative of a test compound that increases the biological persistence of a BoNT/A (¶¶ 24, 55 and 56).

VI. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

1. Whether the Examiner erred in rejecting Claims 3, 4, 46 and 47 as allegedly lacking written description pursuant to 35 U.S.C. § 112, ¶ 1 where the Examiner erred in considering the amended material as new matter because amendment support was found in U.S. Patent Application 10/757,077 which was incorporated by reference in the present specification.

2. Whether the Examiner erred in rejecting Claims 1, 3-20, 22, 45-47, 56 and 57 as allegedly being obvious pursuant to 35 U.S.C. § 103(a) over the Schmidt patent in view of the Fernandez-Salas I abstract and the Fernandez-Salas II abstract, where the Examiner has failed to make a *prima facie* case by establishing by a preponderance of evidence that 1) the cited prior art taught each and every claim limitation of the presently claimed method, 2) the cited prior art provided a suggestion or motivation to combining its teaching to achieve the presently claimed method, and 3) there was no reasonable expectation of success to combine the cited prior art to achieve the presently claimed method.

VII. ARGUMENT

A. Cited Art

1. The Schmidt patent.

James J. Schmidt and Robert G. Stafford, *High Throughput Assays for the Proteolytic Activities of Clostridium Neurotoxins*, U.S. Patent 6,762,280 (effective filing date Sep. 25, 2000), hereafter the "Schmidt patent."

2. The Fernandez-Salas I abstract.

Ester Fernandez-Salas et al., *Plasma Membrane Localization Signals in the Light Chain of Botulinum Neurotoxin Serotype A*, ABS 9.2 Soc. Neurosci. Abstr. Viewer Itiner. (Nov., 2003), hereafter the "Fernandez-Salas I abstract."

3. The Fernandez-Salas II abstract.

Ester Fernandez-Salas et al., *Localization of BoNT Light Chains in Neuronal and Non-Neuronal Cell Lines, Implications for the Duration of Action of the Different Serotypes*, 365(Suppl. 2) Naunyn-Schmiedeberg Arch. Pharmacol. ABS R19 (Jun., 2002), hereafter the "Fernandez-Salas II abstract."

B. The Examiner erred in rejecting Claims 3, 4, 46 and 47 as allegedly lacking written description pursuant to 35 U.S.C. § 112, ¶ 1.

The Examiner has rejected Claims 3, 4, 46 and 47 as allegedly lacking written description pursuant to 35 U.S.C. § 112, ¶ 1. The Examiner contends that the amended claim limitations "about 20% to about 300% increase" and "about 10% to about 90% reduction" did not appear in the specification or original claims, as filed, and thus these recited limitations constitute new matter absent evidence for their support. See, March 29, 2007 Office Action at p. 15, ¶ 1, lines 8-17.

1. The Examiner erred in maintaining that the incorporation by reference of U.S. Patent Application Serial No. 10/757,077 constitutes new matter.

The Examiner asserts that "the recitation of [U.S. Patent Application Serial No. 10/757,077] as incorporated by reference in the amended first paragraph was not found in the original specification [and as such] would be considered as new matter into the disclosure." See, March 29, 2007 Office Action at p. 8, ¶ 3, lines 2-5. Thus, the first question is whether U.S. Patent Application Serial No. 10/757,077 was properly incorporated by reference in the present specification.

According to *MPEP* § 608.01(p), a referencing application must include an identification of the referenced patent, application, or publication being incorporated by reference. Although reference to an attorney's docket number and filing date for an incorporated reference is desirable, any description in the specification reasonably identifying the subject matter contained in the incorporated reference will be sufficient to establish identity for that incorporated reference. *Ex parte Harvey*, 163 USPQ 572 (B.P.A.I. 1968); and *In re Fouche*, 439 F.2d 1237 (C.C.P.A. 1971). *MPEP* § 608.01(p) acknowledges this precedent by stating that "Guidelines for situations where applicant is permitted to fill in a number for Application No. _____ left blank in the application as filed can be found in *In re Fouche*, 439 F.2d 1237, 169 USPQ 429 (CCPA 1971)." Thus, any description reasonably describing the subject matter of an incorporated reference will establish its identity.

In *Ex parte Harvey*, the applicant wanted to amend the specification by introducing the serial number and filing date of two copending applications referred to in the specification merely as "copending applications." *Ex parte Harvey*, 163 USPQ at 573. The Examiner rejected this amendment as new matter under 35 U.S.C. 132(a) on the ground that the incorporated references were insufficiently identified. *Id.* The Board reversed, finding that "[t]he description of the subject matter of the two applications and their relation to the claimed subject left little doubt . . . of the identity of the applications." *Id.* The Board further indicated that, although not required, unambiguous identification of an incorporated application such as citation to an attorney's docket number and application filing date was desirable. *Id.*

Likewise, in *In re Fouche*, the Federal Circuit held that identification of an incorporated reference need only be reasonably precise. *In re Fouche*, 439 F.2d at 1089. In this case, an applicant disclosed in a pending application that a claimed compound could be "prepared as described in Example I of our application No." *Id.* at 1087. The specification provided no other identification of the referenced application. *Id.* The Board affirmed an Examiner's new matter rejection when the applicant attempted to amend the specification to "my Application Serial No. 459,921 filed May 17, 1965," ruling that "our application No" did not uniquely identify the incorporated reference and the applicant could have used more precise language. *Id.* at 1089. However, the *Fouche* Court reversed, finding that an applicant should be allowed to incorporate the disclosure of a reference "so long as the reference application is sufficiently well identified to distinguish it from all others." *Id.* at 1088. The Court also affirmed the Board's ruling in *ex parte Harvey*, stating that sufficient identification of an incorporated reference does not necessarily require an attorney's docket number. *Id.*

Thus, an incorporated reference need only be reasonably identified to distinguish it from other references, and although not required, an attorney's docket number is considered unambiguous identification of the incorporated reference.

The original first paragraph of the present application was:

> This application is a continuation-in-part of U.S. Application Serial No. _____ [Attorney Docket No. ALLE0014-103] filed January 14, 2004, which is a continuation-in-part of U.S. Application Serial No. 10/163,106 filed June 4, 2002, which is a continuation-in-part of U.S. Application Serial No. 09/910,346 filed July 20, 2001; which is a continuation-in-part U.S. Application Serial No. 09/620,840 filed July 21, 2000, the disclosures of which are incorporated herein by reference in their entirety.

The marked up version of the amended first paragraph of the present application is:

> The disclosures of ~~This application is a continuation-in-part of~~ U.S. Application Serial No. 10/757,077 ~~_____ [Attorney Docket No. ALLE0014-103]~~ filed January 14, 2004, ~~which~~

~~is a continuation-in-part of~~ U.S. Application Serial No. 10/163,106 filed June 4, 2002, ~~which is a continuation-in-part of~~ U.S. Application Serial No. 09/910,346 filed July 20, 2001 and ~~; which is a continuation-in-part~~ U.S. Patent No. 6,903,187 issued June 7, 2005 ~~Application Serial No. 09/620,840 filed July 21, 2000, the disclosures of which~~ are all incorporated herein by reference in their entirety.

The clean version of the amended first paragraph of the present application is:

The disclosures of U.S. Application Serial No. 10/757,077 filed January 14, 2004, U.S. Application Serial No. 10/163,106 filed June 4, 2002, U.S. Application Serial No. 09/910,346 filed July 20, 2001 and U.S. Patent No. 6,903,187 issued June 7, 2005 are all incorporated herein by reference in their entirety.

The Applicants respectfully submit that Attorney Docket No. ALLE0014-103 filed January 14, 2004 is U.S. Application Serial No. 10/757,077. The original disclosure indicates that the incorporated reference is a continuation-in-part application that was filed on January 14, 2004 under the attorney's docket No. ALLE0014-103. Thus, like *Ex parte Harvey* the mere fact that the description of the subject matter of the incorporated application and its relation to the claimed subject matter of the present specification leaves little doubt to the identity of the incorporated application. Furthermore, merely viewing patent application 10/757,077 in PAIR and examining the pdf file for the specification filed on January 14, 2004 reveals that the attorney's docket No. ALLE0014-103 is clearly present in the header on each page of the specification. For the Board's convenience, the Applicants have provided a copy of page 1 from U.S. Application Serial No. 10/757,077. See Appendix A. As indicated in both *Ex parte Harvey* and *In re Fouche*, reference to the incorporated application by both an attorney's docket number and its filing date is definite and unambiguous identification of the incorporated reference. As such, the evidence of record clearly establishes the patent application referred to as "Attorney Docket No. ALLE0014-103 filed January 14, 2004" in the first paragraph of the present specification is U.S. Application Serial No. 10/757,077.

The Examiner contends that U.S. Application Serial No. 10/757,077 was improperly incorporated by reference because "the specification fails to indicate the relationship between

the instant application and the cited application. See. *MPEP* § 201.01 (II)." See, July 23, 2007 Advisory Action at ¶ 2, lines 4-8. *MPEP* § 201.11 covers aspects of claiming the benefit of an earlier filing date under 35 U.S.C. §§ 120 and 119(e). However, the question is whether U.S. Application Serial No. 10/757,077 was properly incorporated by reference and not whether the present specification properly claims benefit of an earlier filed application. As such, the Examiners contention is groundless and without merit.

Thus, the Examiner has erred in maintaining that the incorporation by reference of U.S. Application Serial No. 10/757,077 was not found in the original specification because 1) the original specification clearly indicates that Attorney Docket No. ALLE0014-103 filed January 14, 2004, which is a continuation-in-part of U.S. Application Serial No. 10/163,106 filed June 4, 2002 was incorporated by reference in its entirety; and 2) a preponderance of the evidence of record unambiguous indicates that Attorney Docket No. ALLE0014-103 is U.S. Application Serial No. 10/757,077.

2. The Examiner erred in maintaining the new matter rejection because the present specification in its entirety provides adequate written description support for Claims 3, 4, 46 and 47.

The second question is whether, when viewed in its entirety, the present specification provides sufficient written description support for Claims 3, 4, 46 and 47 as presently amended.

According to *MPEP* § 2163, to satisfy the written description requirement, a patent specification must describe the claimed invention in sufficient detail that a person of ordinary skill in the art can reasonably conclude that the inventor had possession of the claimed invention. The subject matter of the claim need not be described literally (*i.e.*, using the same terms or *in haec verba*) in order for the disclosure to satisfy the description requirement. *Id.* Possession of the claimed subject matter can be shown through express, implicit, or inherent disclosure. *Id.* The Examiner erred in maintaining the 35 U.S.C. § 112, ¶ 1 written description rejection against Claims 3, 4, 46 and 47 because the amended claim limitations "about 20% to about 300% increase" and "about 10% to about 90% reduction" are described in the present specification

with sufficient detail that a person of ordinary skill in the art would reasonably conclude that the Applicants had possession of the claimed invention.

The presently claimed method is directed towards a method of identifying a compound that either reduces or increases a biological persistence of a BoNT/A. Claims 3 and 46 indicate that the claimed method identifies a compound that increases biological persistence by observing if the compound causes an about 20% to about 300% more BoNT/A light chain localization to plasma membrane over time relative to a control. Claims 4 and 47 indicate that the claimed method identifies a compound that reduces biological persistence by observing if the compound causes an about 10% to about 90% reduction in BoNT/A light chain localization to the plasma membrane over time relative to a control.

First, the present specification teaches that a compound that decreases the characteristic localization pattern of BoNT/A decreases biological persistence of that toxin, whereas a compound that increases the localization pattern of BoNT/A increases biological persistence of that toxin. For example, the present specification teaches that the duration of action of botulinum neurotoxins is related to their subcellular localization, with plasma localization resulting in longer duration. See, *e.g.*, Present specification at ¶ 17. The present specification at ¶ 23 states that "[a] change in the localization pattern of the light chain in the cell following contacting the cell with the test compound indicates that the test compound alters, *i.e.*, inhibits or enhances, the biological persistence of the toxin." The present specification further elaborates on the relationship between biological persistence of a BoNT/A light chain and it localization to a membrane in ¶¶ 24-25; and 52-53 where it discloses that compounds that disrupt membrane localization of a toxin light chain result in reduced biological persistence, whereas compounds that enhance membrane localization of the toxin light chain result in increased biological persistence. Lastly, Example 6 discloses methods for identifying compounds that alter the biological persistence of a Clostridial toxin.

Second, the [present specification incorporated by reference in its entirety U.S. Patent Application Serial No. 10/757,077, hereafter the "077 Application." See, *Id.* at ¶ 1. The 077 Application discloses, in part, modified BoNT/As that have increased biological persistence and modified BoNT/As that have decreased biological persistence. See, *e.g.*, the 077 Application at

¶¶ 109 and 130. For example, the Application 077 indicate that a modified neurotoxin can have an increased biological persistence of about 20% to about 300% longer than a neurotoxin that is not modified. *Id.* at ¶¶ 109, 221, 225 and 285 of. Similarly, this application indicates that a modified neurotoxin can have a decreased a biological persistence of about 10% to about 90% that of an unmodified BoNT/A light chain. *Id.* at ¶ 156. In addition, this application indicates that the key factor in determining the biological persistence is the localization of BoNT/A light chain to the cellular membrane because the cell membrane appears to protect the light chain from intracellular protein degradation.. *Id.* at ¶¶ 133 and 142. Furthermore, the 077 Application discloses that SNAP-25, the substrate for BoNT/A activity, is localized to the plasma membrane. *Id.* at ¶¶ 137, 321, and FIG 5.

Thus, taken in its entirety, the present specification describes with sufficient detail the fact that 1) both BoNT/A light chain and its substrate SNAP-25 are localized to the plasma membrane; 2) localization of the BoNT/A light chain is necessary for the light chain to cleave SNAP-25; 3) the length of time a BoNT/A light chain remains associated with the plasma membrane is directly correlated with it biological persistence; 4) the biological persistence of a modified BoNT/A light chain can be increased by about 20% to about 300%; 5) the biological persistence of a modified BoNT/A light chain can be decrease by about 10% to about 90%; and 6) the increased or decreased biological persistence of a modified BoNT/A light chain directly correlates with a corresponding increase or decrease of light chain localization to a plasma membrane; 7) the disclosed screening methods use this altered plasma membrane localization as a read-out of the effects a compound has on biological persistence; 8) a compound that increases biological persistence can be identified by it causing an increased localization of a BoNT/A light chain to the plasma membrane over time; and 9) a compound that decreases biological persistence can be identified by it causing an reduced localization of a BoNT/A light chain to the plasma membrane over time.

As such, a person of ordinary skill in the art would reasonably conclude that since a modified BoNT/A light chain can have an increased biological persistence of about 20% to about 300%, then a compound that that increases biological persistence could have a similar effect because both the internal modification and the external influence would affect the light chain in the same manner, namely affecting its ability to associate with the plasma membrane. Similarly, such a

person would understand that since a modified BoNT/A light chain can have an reduced biological persistence of about 10% to about 90%, then a compound that that decreases biological persistence could have a similar effect because both internal modification and external influences would similarly affect the ability of the light chain to associate with the plasma membrane.

Thus, a person of ordinary skill in the art would reasonably conclude that the present specification describes with sufficient detail the fact the present specification describes with sufficient detail 1) a method of identifying a compound that increases a biological persistence of a BoNT/A by observing an about 20% to about 300% more BoNT/A light chain localized to the plasma membrane as compared to a control; and 2) a method of identifying a compound that decreases a biological persistence of a BoNT/A by observing an about 10% to about 90% reduction in plasma membrane localization of the BoNT/A light chain as compared to a control.

The Examiner asserts that the claim amendments lack written description support because the original "specification fails to point to specific portions of the document where the subject matter being incorporated may be found. Mere reference to another application, patent, or publication is not an incorporation of anything therein into the application containing such reference for purpose of the disclosure required by 35 U.S.C. 112, first paragraph. *In re de Seversky*, 474 F.2d 671, 177 USPQ 144 (CCPA 1973)." *See,* July 23, 2007 Advisory Action, p. 2, ¶ 2, lines 1-4. However, the Examiner's assertion fails on several accounts.

First, the Examiner's interpretation of *In re de Seversky* is an incorrect mischaracterization of its holding. In this appeal, de Seversky, the applicant, argued that merely indicating that a later filed application was a "continuation in part" of an earlier filed application was ipso facto, an "incorporated by reference" of that disclosure. *In re de Seversky*, 474 F.2d 671 at 674. The Federal Circuit disagreed, holding that mere reference to an earlier filed application as a "continuation-in-part" did not operate as language sufficient to incorporate by reference any part of the specification of the earlier filed application. *Id.* The Court explained that "the purpose of "incorporation by reference" is to make one document become a part of another document by referring to the former in the latter in such a manner that it is apparent that the cited document is part of the referencing document as if it were fully set out therein." *Id.*

Unlike *de Seversky*, the Applicants are not merely referring to U.S. Application Serial No. 10/757,077 as a "continuation in part" of the present application. *See, supra,* pp. 6-9. The Applicants have explicitly used the "incorporation by reference" language when referring to this earlier filed application. As such, the Examiner has erred in asserting that the Applicants are merely referring to another patent application.

Second, the Examiner's position is contrary to both judicial precedent and USPTO policy. For example, the Custom Court of Patents and Appeals stated that "As the expression itself implies, the purpose of "incorporation by reference" is to make one document become a part of another document by referring to the former in the latter in such a manner that it is apparent that the cited document is part of the referencing document as if it were fully set out therein." *In re Lund*, 376 F.2d 982, 989, (C.C.P.A. 1967). Likewise, the USPTO stressed that "an applicant may incorporate by reference the prior application by including, in the continuing application-as-filed, a statement that such specifically enumerated prior application or applications are 'hereby incorporated herein by reference.' The inclusion of this incorporation by reference of the prior application(s) will permit an applicant to amend the continuing application to include any subject matter in such prior application(s), without the need for a petition." See, *e.g.*, Department of Commerce, Patent and Trademark Office, Changes to Patent Practice and Procedure, 62 Fed. Reg. 53132, 53146 (Oct. 10, 1997). Furthermore, according to *MPEP* § 2163.07(b): "Instead of repeating some information contained in another document, an application may attempt to incorporate the content of another document or part thereof by reference to the document in the text of the specification. The information incorporated is as much a part of the application as filed as if the text was repeated in the application, and should be treated as part of the text of the application as filed. Replacing the identified material incorporated by reference with the actual text is not new matter."

As such, the Examiner's contention that merely refers to another application and does not incorporate anything is groundless and unsubstantiated by any evidence. Thus, the Applicants respectfully submit that there is adequate written description support for the recited limitations claimed because the present specification conveys with reasonable clarity to those skilled in the art that, as of the filing date sought, that the Applicants were in possession of the claimed

method of identifying a compound that either reduces or increases a biological persistence of a BoNT/A.

3. The Examiner erred by failing to make a prima facie case for a lack of written description against Claims 3, 4, 46 and 47.

The Examiner contends that "[s]upport is not found for the limitations of about 20% to about 300% increase and about 10% to about 90% reduction as disclosed in the original specification and thus the recitations constitute new matter absence evidence for their support." October 3, 2006 Office Action at p. 15, ¶ 1, lines 12-14. Thus, the question is whether the Examiner has made of record evidence sufficient to establish a *prima facie* case of lack of written description.

According to *MPEP* § 2163.04, the Examiner "has the initial burden of presenting by a preponderance of evidence why a person skilled in the art would not recognize in an applicant's disclosure a description of the invention defined by the claims." The burden fails on the Examiner because the disclosure of a specification "is presumed to be adequate, unless or until sufficient evidence or reasoning to the contrary has been presented by the examiner to rebut the presumption." *Id.* "The examiner, therefore, must have a reasonable basis to challenge the adequacy of the written description." *Id.*

The only line of reasoning to support a *prima facie* case of lack of written description offered by the Examiner was that the claimed range of increase or reduction was different from the original disclosure. For example, the Examiner asserts a lack of written description on the ground that the present specification "fails to disclose about 20% to about 300% increase as recited in claims 3 and 46, and about 10% to about 90% reduction as recited in claims 4 and 47." October 3, 2006 Office Action at p. 14, ¶ 3, lines 1-3. Further, the Examiner has maintained this rejection on the assertion that the 077 Application was not incorporated by reference in the original disclosure. As discussed above, this basis for the rejection is in error because the incorporation by reference of U.S. Application Serial No. 10/757,077 was proper and, when viewed in its entirety, the present specification provides adequate written description support for amended Claims 3, 4, 46 and 47. *See, supra,* pp. 6-14.

As such, the Examiner has failed to substantiate a *prima facie* case of lack of written description for the amended claim limitations "about 20% to about 300% increase" and "about 10% to about 90% reduction" because 1) the Examiner's refusal to recognize the incorporation by reference of U.S. Application Serial No. 10/757,077 is unfounded and without merit; and 2) the present specification in its entirety provides sufficient written description support for the claim amendments. Thus, the Examiner erred, and the Board should therefore reverse the Examiner's 35 U.S.C. § 112, ¶ 1 written description rejections against Claims 3, 4, 46 and 47.

C. The Examiner erred in rejecting Claims 1, 3-20, 22, 45-47, 56 and 57 as allegedly being obvious pursuant to 35 U.S.C. § 103(a) over the Schmidt patent in view of the Fernandez-Salas I abstract and the Fernandez-Salas II abstract.

1. The Examiner failed to establish a *prima facie* case of obviousness against Claims 1, 3-20, 22, 45-47, 56 and 57.

The Examiner has rejected Claims 1, 3-20, 22, 45-47, 56 and 57 as allegedly being obvious pursuant to 35 U.S.C. § 103(a) over the Schmidt patent in view of the Fernandez-Salas I abstract and the Fernandez-Salas II abstract. Specifically, the Examiner argued that:

> [i]t would have been obvious for one of ordinary skill in the art at the time the instant invention was made to combine the teachings of [the Schmidt patent and the Fernandez-Salas I abstract] to screen a compound that affects a biological persistence of a Clostridial toxin by evaluating the localization and enzymatic activity. The person of ordinary skill in the art would have been motivated to make those modifications because the persistence of proteolytic activity of a Clostridial toxin relies on the light chain domain of the toxin. In addition, the specific substrates of BoNT/A is SNAP-25, which is one of SNARE complex involved in exocytosis of synaptic vesicles. A Clostridial toxin is composed of one heavy chain and one light chain. The interaction of BoNT/A with its substrate, such as SNAP-25, is intracellular cleavage of SNAP-25 and subsequently inhibits exocytosis of synaptic vesicles. The internalization of BoNT/A is to bring the molecule to interact with SNAP-25. Therefore, one of ordinary skill in the art would have expected success in screening a compound that alters/inhibits a biological persistence of

a Clostridial toxin by contacting cells expressing a light chain to a Clostridial toxin and evaluating the localization and proteolytic activity of the light chain." See, May 26, 2006 Office Action at p. 20, ¶ 3, line 1 through p. 21, ¶ 1, line 12.

The issue is whether the Examiner erred in rejecting the presently claimed method as allegedly being obvious under 35 U.S.C. § 103(a) over the cited prior art on the ground that the Examiner failed to establish a *prima facie* case. To establish a *prima facie* case of obviousness, the Examiner must show that 1) the prior art taught or suggested all the limitations of the claimed invention; 2) the prior art provided some suggestion or motivation to modify the invention disclosed in the cited reference(s) or to combine the teachings of the cited reference(s) to create the claimed invention; and 3) there was a reasonable expectation of success for making the proposed modification or combination. *MPEP*, § 2142.

The Examiner bears the initial burden of factually supporting any *prima facie* conclusion of obviousness. *Id*. As such, the Examiner must establish each of these elements by a preponderance of evidence standard by providing "evidence which as a whole shows that the legal determination sought to be proved . . . is more probable than not." *Id.* If the Examiner fails to establish a *prima facie* case, then the claimed invention is not obvious. *Id*. In determining if a combination of old elements is non-obvious, the Examiner must assess whether, in fact, a person of ordinary skill in the art at the time of the invention, confronted by the same problems facing the inventor, with no knowledge of the claimed invention, would have been led to selected the various elements from the prior art and combined them in the manner claimed. *Cross Medical Products, Inc. v. Medtronic Sofamor Danek, Inc.*, 424 F.3d 1293, 1321 (Fed. Cir. 2005); *In re Kahn*, 441 F.3d 977, 988 (Fed. Cir. 2006).

That particular finding of objective facts relating to the desirability to combine the prior art teaching must be vigorously enforced because "[t]he suggestion to combine requirement stands as a critical safeguard against hindsight analysis and rote application of the legal test for obviousness." *In re Rouffet*, 149 F.3d 1350, 1358 (Fed. Cir. 1998). Likewise, the Federal Circuit has found that "the best defense against the subtle but powerful attraction of a hindsight-based obviousness analysis is rigorous application of the requirement for a showing of teaching or motivation to combine prior art references." *In re Lee*, 277 F.3d 1338, 1343 (Fed. Cir. 2002).

In fact, the Federal Circuit has indicated that the legal determination of obviousness "should be based on evidence rather than on mere speculation or conjecture" and that "[b]road conclusory statements standing alone are not evidence." *In re Kotzab*, 217 F.3d 1365, 1370 (Fed. Cir. 2000); *Alza Corp. v. Mylan Laboratories, Inc.*, 464 F.3d 1286 1290 (Fed. Cir. 2006). The Federal Circuit has warned that "[c]lose adherence to this methodology is especially important in cases where the very ease with which the invention can be understood may prompt one to fall victim to the insidious effect of a hindsight syndrome wherein that which only the invention taught is used against the teacher." *Kotzab*, 217 F.3d at 1369 (internal quotes deleted).

The Examiner has failed to produce sufficient evidence that establishes all of these elements, and the Board should therefore reverse the Examiner's obvious rejections under 35 U.S.C. § 103(a) against Claims 1, 3-20, 22, 45-47, 56 and 57.

a. The Examiner failed to establish by a preponderance of evidence that the cited prior art taught each and every claim limitation of the presently claimed method.

To establish *prima facie* obviousness of a claimed invention, all the claim limitations must be taught or suggested by the prior art because the claimed invention must be considered as a whole. *MPEP* § 2143; § 2143.03. The Examiner erred because it would not be obvious to modify or combined the cited prior art to develop the presently claimed method because the cited art does not teach or suggest 1) any method of identifying a compound that alters biological persistence; let alone 2) any method of identifying a compound that alters biological persistence by observing altered light chain localization patterns to the plasma membrane.

1) The Examiner erred by incorrectly equating biological persistence as proteolytic activity and failing to identify the limitation of a compound that alters biological persistence by observing BoNT/A plasma membrane localization patterns relative to a control as presently claimed.

The Examiner contends that the teaching of the Schmidt patent meets "the limitations of screening for a compound that reduces/increases a biological persistence of BoNT/A by detecting proteolytic activity of BoNT/A light chain on SNAP-25 or other substrates of SNARE

proteins." October 3, 2006 Office Action at p. 12, ¶ 1, lines 12-14. The Examiner supports this contention by indicating that the Schmidt patent "teaches a method for identifying a compound that inhibits/enhances the proteolytic activity of botulinum neurotoxin serotype A (BoNT/A) by incubating neurotoxin with the test compound and a fluorescence labeled substrate, and measuring the fluorescence signal resulting from proteolytic cleavage of the substrate by neurotoxin. [The Schmidt patent] teaches using an FRET assay of detecting the proteolytic activity of the light chain of BoNT/A and a ELISA method to screen a compound that inhibits/enhances the proteolytic activity of the light chain of BoNT/A." *Id.* at lines 4-11. However, the contention the limitations of screening for a compound that reduces/increases a biological persistence of BoNT/A by detecting proteolytic activity of BoNT/A light chain on SNAP-25 or other substrates of SNARE proteins is factually incorrect because 1) this is not the claimed limitation; 2) the Examiner has failed to consider the present specification in its entirety; 3) the Examiner has failed to consider the Schmidt patent in its entirety; 4) the Examiner has failed to establish by a preponderance of evidence that the claim limitation actually claimed by the Applicants and the claim limitation asserted by the Examiner are the same limitation; and 5) the Fernandez-Salas II abstract also supports the position that biological persistence and proteolytic activity are separate and distinct properties of BoNT/A functionality.

First, the Examiner erred by mischaracterizing the presently claimed method by failing to consider the present claimed method in its entirety. The presently claimed methods identify compound that increase/decrease biological persistence by observing and comparing BoNT/A light chain membrane localization pattern to a membrane localization pattern of a BoNT/A light chain in a cell in an absence of the test compound. *See, infra,* p. 45, Claim 1. As such, a compound altering biological persistence is identified by detecting alterations in the length of time that a light chain remains associated with the plasma membrane. Thus, the Examiner's asserts that the presently claimed methods of identifying a compound that alters BoNT/A biological persistence recite a limitation of detecting proteolytic activity of BoNT/A light chain on SNAP-25 or other substrates of SNARE proteins, is factually incorrect. As such, the Examiner has mischaracterized the presently claimed methods because altered biological persistence is detected by alter membrane localization patterns and not altered proteolytic activity.

Second, the Examiner has erred by failing to consider the present specification in its entirety because this disclosure clearly indicates that biological persistence and proteolytic activity are distinct and separate properties of BoNT/A functionality. The present specification defines biological persistence as "the continuous period of time that a light chain retains its enzymatic activity when that light chain is within a cell or outside of a cell." *See,* Present Specification at ¶ 34. In addition, the use of the term "biological persistence" in the present specification clearly indicates that biological persistence and proteolytic activity are distinct and independent characteristics of the modified toxins disclosed. For instance, the present specification indicates that the discloses methods can identify compounds that 1) reduce biological persistence (*id.* at ¶ 52); 2) enhance biological persistence (*id.* at ¶ 53); 3) inhibit enzymatic activity (see, *id.* at ¶ 54); 4) enhance enzymatic activity (*id.* at ¶ 54); 5) reduce biological persistence and inhibit enzymatic activity (*id.* at ¶ 55); or 6) enhance biological persistence and enhance enzymatic activity (*id.* at ¶ 55).

The present specification further illustrates that biological persistence is a distinct and separate property from proteolytic activity in the types of assays described. For example, the present specification discloses "methods of identifying compounds that alter, *i.e.*, enhance or inhibit, the biological persistence and enzymatic activity of a Clostridial toxin. The methods comprise performing both a test localization assay and a test enzymatic assay using a test compound. The assays may be run in either order or simultaneously." *Id.* at ¶ 26. The test localization assay, which is presently claimed, "comprises the steps of contacting a cell that comprises a Clostridial toxin light chain with a test compound and determining whether the localization pattern of the light chain in the cell differs following contacting the cell with the test compound compared to the localization pattern of the light chain in the cell in the absence of the test compound." *Id.* at ¶ 22. On the other hand, the test enzymatic assay "comprises contacting a sample containing the light chain of the toxin with an enzymatic substrate of the light chain in the presence of the test compound, and determining whether the substrate is processed by the light chain into enzymatic product." *Id.* at ¶ 25.

Thus, the present specification clearly indicates that biological persistence and proteolytic activity are distinct and independent characteristics of BoNT/A functionality and each

characteristic can be assayed separately using the methods disclosed in the present specification.

Third, the Examiner has erred by failing to consider the disclosure of the Schmidt patent in its entirety because this patent merely discloses an assay that measure only proteolytic activity. The Schmidt patent discloses an in vitro substrate-release assay useful for identifying a compound that inhibits or enhances the proteolytic activity of BoNT/A, see, col. 9, line 65 through col. 10, line12; and Claim 9. In this method, a botulinum toxin is "incubated with a test compound . . . transferred to solid supports onto which is immobilized a peptide substrate for the BoNT enzyme being tested . . . and processed," see col. 10, lines 1-5. The immobilized peptide substrate is a SNARE peptide that contains a reactive group at its C-terminal end used to attach the substrate to the solid support, a fluorophore group at its N-terminal end used in the fluorescence detection method, and an intervening BoNT cleavage site, col. 7, lines 15-20; and col. 7, lines 26-56. The Schmidt patent indicates that "a reduction in the ability of the toxin to cleave the peptide substrate relative to unincubated toxin indicates a[n] inhibitory compound," see, col. 10, lines 6-9. On the other hand, "an increase in the ability of the toxin to cleave the peptide substrate relative to unincubated toxin indicated a stimulatory compound," see col. 10, lines 9-12. As such, the Schmidt patent fails to teach or suggest any concept of biological persistence, let alone a method of identify compound that alter BoNT/A biological persistence. Thus, it is clear that the Schmidt patent only discloses an assay that can screen for compounds that alter the proteolytic activity of a BoNT/A.

Fourth, the Examiner has erred by failing to provide any evidence or line of reasoning indicating how a person of ordinary skill in the art would interpret BoNT/A proteolytic activity and BoNT/A biological persistence as defining the same property. The Examiner has merely asserted that the Schmidt patent teaches a method of screening for a compound that reduces/increases a biological persistence of BoNT/A because this patent discloses a method of screening for a compound that reduces/increases proteolytic activity of BoNT/A. However, this assertion is a conclusory statement based purely the Examiner's opinion and mere speculation. As such, the Examiner has failed to submit any evidence or reasoning based on particular findings of fact that biological persistence and proteolytic activity are one in the same characteristic.

As such, the Examiner assertion that the Schmidt patent teaches the limitation of screening for a compound that reduces/increases a biological persistence of BoNT/A by detecting proteolytic activity of BoNT/A light chain on SNAP-25 or other substrates of SNARE proteins is unfounded and without merit because the Examiner failed to consider both the presently claimed method and the Schmidt patent as a whole. The Examiner mischaracterized the Schmidt patent assay by asserting that this assay was capable of measuring biological persistence because 1) biological persistence and proteolytic activity are separate and distinct properties; 2) the presently claimed method identify compounds that altered BoNT/A biological persistence by detecting altered membrane localization patterns of BoNT/A light chain and not altered proteolytic activity; and 3) the Schmidt patent assay can only identify compounds that alter BoNT/A proteolytic activity.

The Fernandez-Salas I abstract and Fernandez-Salas II abstract are completely silent with respect to 1) the limitations of identifying a compound that alters biological persistence by observing altered proteolytic activity, as asserted by the Examiner; let alone 2) the limitations of identifying a compound that alters biological persistence by observing BoNT/A plasma membrane localization patterns relative to a control as presently claimed. In fact, the teaching of the Fernandez-Salas II abstract further underscores the fact that biological persistence and proteolytic activity are distinct and separate properties. This abstract discloses that in order to investigate the underlying molecular mechanisms of biological persistence, fluorescently-labeled light chains of various botulinum toxins were transformed into cells in order to determine their subcellular localization. Fernandez-Salas II abstract at lines 7-24. On the other hand, proteolytic activity of these light chains was determined by a western blot assay. *Id.* at lines 10-11. As such, the use of subcellular localization to gain insight into biological persistence, but western blot analysis to determine proteolytic activity highlights the fact that these two properties are distinct and separate.

Thus, the Examiner has failed to establish by a preponderance of the evidence that the cited prior art has taught each and every limitation of the presently claimed method because the Schmidt patent, the Fernandez-Salas I abstract and Fernandez-Salas II, either alone or in combination, fail to teach or suggest the limitations of identifying a compound that alters biological persistence by observing BoNT/A plasma membrane localization patterns relative to a

control. Thus, the Examiner erred, and the Board should therefore reverse the Examiner's 35 U.S.C. § 103(a) obviousness rejections against Claims 1, 3-20, 22, 45-47, 56 and 57.

2) The Examiner erred by failing to identify the limitation of a compound that alters biological persistence by observing BoNT/A plasma membrane localization patterns relative to a control as presently claimed.

The Examiner contends that the teaching of the Fernandez-Salas I abstract meets "the limitations of observing the change of localization of light chain of BoNT/A while cleaves SNAP25 since cleavage occurs in intracellular compartment and the interaction of BoNT/A light chain with SNAP25 triggers the endocytosis of the complex." October 3, 2006 Office Action at p. 12, ¶ 1, lines 18-21. The Examiner supports this contention by indicating that the Fernandez-Salas I abstract teaches that "the fusion protein of the light chain of BoNT/A to GFP protein is colocalized with SNAP25 when transfected in neurons. The colocalization can be detected by confocal microscopy." *Id.* at lines 15-17.

First, the Examiner has erred by mischaracterizing the presently claimed method by failing to consider the present claimed method in its entirety. The Examiner's assertion that the is factually incorrect because 1) BoNT/A biological persistence is controlled by light chain localization to the plasma membrane; 2) BoNT/A biological persistence is independent from and unrelated to whether the light chain can cleave SNAP-25; and 3) the fact that SNAP-25 cleavage occurs intracellularly is immaterial to the claimed limitation of observing and comparing light chain membrane localization patterns in order to determine altered BoNT/A biological persistence. As such, the Examiner has utterly mischaracterized the presently claimed methods by asserting that the claimed limitation is "observing the change of localization of light chain of BoNT/A while cleaves SNAP25 since cleavage occurs in intracellular compartment and the interaction of BoNT/A light chain and SNAP-25 triggers endocytosis of the complex. As such, the Examiner has failed to establish that the Fernandez-Salas I abstract suggests or teaches the limitations as presently claimed.

As a first point, identification of a compound that alters BoNT/A biological persistence is based upon the length of time a BoNT/A light chain remains associated with the plasma membrane.

The presently claimed method is based on alteration of BoNT/A membrane localization patterns and not BoNT/A proteolytic activity. Step (b) of the claimed limitation recites "observing the membrane localization pattern of the BoNT/A light chain following contact of the cell with the test compound." *See, infra,* p. 45, Claim 1. Step (c) recites "comparing the observed BoNT/A light chain membrane localization pattern to a membrane localization pattern of a BoNT/A light chain in a cell in an absence of the test compound." *Id.* The presently claimed method can identify a compound that increases BoNT/A biological persistence by detecting whether a test compound causes an enhanced membrane localization pattern of the BoNT/A light chain over time in a cell exposed to the compound relative to the BoNT/A light chain membrane localization pattern in the cell not exposed to the test compound. *See, e.g.,* Present specification at ¶ 53; and *see, infra*, p. 45, Claim 1. Similarly, the presently claimed method can identify a compound that decreases BoNT/A biological persistence by detecting whether a test compound causes a reduced membrane localization pattern of the BoNT/A light chain over time in a cell exposed to the compound relative to the BoNT/A light chain membrane localization pattern in the cell not exposed to the test compound. *See, e.g.,* Present specification at ¶ 52; and *see, infra*, p. 45, Claim 1. Thus, the presently claimed method determines whether a compound can alter BoNT/A biological persistence by observing whether the amount of time that the BoNT/A light chain remains associated with the plasma membrane is altered when the cell is exposed to a test compound.

As a second point, identification of a compound that alters BoNT/A biological persistence is unrelated to whether the light chain can cleave SNAP-25. As discussed above, biological persistence defines a characteristic that is a distinct and independent from proteolytic activity. *See, supra,* pp. 18-19. As also outlined above, the basis for identifying whether a compound alters BoNT/A biological persistence is whether that compound changes BoNT/A light chain plasma membrane localization patterns in a cell when compared to the light chain membrane patterns in a cell not treated with the test compound. *See, supra,* pp. 22-23. Whether or not the BoNT/A light chain is proteolytic active is inconsequential to how the presently claimed method operates. As such, the Examiner's explanation that the BoNT/A light chain can cleave SNAP-25 is irrelevant to the claimed limitations of observing and comparing BoNT/A light chain localization to the plasma membrane as a means for detecting alter biological persistence. Thus, the Examiner has failed to articulate any reason based on particular findings of fact

supporting the assertion that the Fernandez-Salas I abstract taught or suggested the claim limitation.

As a third point, the Examiner has misconstrued the presently claimed method by factually mischaracterizing how the method operates. For example, the association of BoNT/A with SNAP-25, or cleavage of SNAP-25 by BoNT/A, does not trigger endocytotsis of the complex as asserted by the Examiner. As discussed above, at the time of filing the present specification, it was known to a person of ordinary skill in the art that this intoxication mechanism was a multi-step process. *See, supra,* p. 2. This process is initiated when the BoNT/A binds to BoNT/A-specific receptor complexes present on the plasma membrane surface of a target cell (receptor binding step). Once bound, the toxin/receptor complexes are internalized by endocytosis and the internalized vesicles are sorted to specific intracellular routes (toxin internalization). While in the vesicle, the light chain is separated from the heavy chain, and through a series of events involving the translocation domain, the separated BoNT/A light chain is released from the intracellular vesicle into the cytosol (light chain translocation step). Once in the cytosol, the light chain localizes to the plasma membrane where it can specifically cleaves its substrate, SNAP-25 (intracellular target action step). Cleavage of SNAP-25 prevents exocytosis of the neurotransmitter, thereby causing paralysis.

The Examiner's assertion that the BoNT/A light chain and SNAP-25 are endocytosed is shear fabrication that is unsupported by any scientific evidence. For example, endocytosis is a complex process that results in the formation of intracellular vesicles. *See, e.g.*, Jeffery M. Besterman and Robert B. Low, *Endocytosis: A Review of Mechanisms and Plasma Membrane Dynamics*, 210 Biochem. J. 1-13 (1983) at 1, col. 1, ¶ 1, lines 1-11. Although endocytotic vesicles are observed when the toxin/receptor complex is internalized (internalization step), there is no evidence indicating, nor has there ever been a suggestion, that a BoNT/A light chain/cleaved SNAP-25 complex is endocytosed (a post intracellular target action step) since intracellular vesicles containing this supposed complex have never been observed. In addition, it was understood by a person of ordinary skill in the art that cleaved SNAP-25 degradation mechanism was responsible for this products removal from the membrane. *See, generally*, James E. Keller et al., *Persistence of Botulinum Neurotoxin Action in Cultured Spinal Cord Cells*, 456 (1) FEBS Lett. 137-142 (1999); Michael Adler et al., *Persistence of Botulinum*

Neurotoxin A Demonstrated by Sequential Administration of Serotypes A and E in Rat EDL Muscle, 39 Toxicon 233-243 (2001); and Patrick G. Foran et al., *Evaluation of the Therapeutic Usefulness of Botulinum Neurotoxin B, C1, E, and F Compared with the Long Lasting Type A*, 278(2) J. Biol. Chem. 1363-1371 (2003). Thus the Examiner's characterization that BoNT/A association/cleavage of SNAP-25 triggers endocytotsis of the complex is unfounded based on current understanding of how the intoxication process works and is contrary to what a person of ordinary skill in the art would have understood to be the mechanism of intoxication.

Furthermore, SNAP-25 cleavage product is eliminated from the plasma membrane in a manner independent of BoNT/A biological persistence. SNAP-25 is rapidly and continuously synthesized and transported into the plasma membrane, with pulse-chase studies demonstrating that this SNARE protein exhibits a turn-over rate of about 5 days. *See, e.g.,* Arleen Loewy et al., *The Major ^{35}S-Methionine-Labeled Rapidly Transported Protein (Superprotein) is Identical to SNAP-25, a Protein of Synaptic Terminals*, 11(11) J. Neurosci. 3412-3421 (1991) at 3414, Figure 2. In addition, pulse-chase experiments indicate that cleaved SNAP-25 exhibits similar degradation rates of approximately 5 days, irrespective of whether this protein is cleaved by BoNT/A or BoNT/E. See, *e.g.*, Keller, 456 (1) FEBS Lett. at 141, col. 2, ¶ 2, line 12 through ¶ 3, line 10. However, this same study indicates that BoNT/A-induced paralysis lasts for approximately 80 days. *Id.* at 139, col. 1, ¶ 2, lines 7-11. In a different study, the half-life of SNAP-25 in cultured central neurons was determined to be about 1 day whereas the half-life of BoNT/A was measured at greater than 31 days. Foran, 278(2) J. Biol. Chem. at Table I and 1367, col. 2, ¶ 1, lines 1-6. Lastly, BoNT/E biological persistence is approximately 4 weeks in treatments for dystonias, whereas BoNT/A biological persistence is about 4 months for such treatments. *Id.* at 1363, col. 2, ¶ 2, lines 1-4.

As such, the Examiner's assertion that BoNT/A light chain/cleaved SNAP-25 complex endocytosis suggests an assay for biological persistence is without merit because cleaved SNAP-25 degradation rates are the same irrespective of whether cleavage was mediated by BoNT/A or BoNT/E. If the Examiner's assertion was correct, one would expect that BoNT/E-cleaved SNAP-25 would have a much faster turnover rate than BoNT/A-cleaved SNAP-25 since BoNT/E has a mush shorter biological persistence. This is in fact, not what has been scientifically demonstrated. BoNT/A-cleaved SNAP-25 and BoNT/E-cleaved SNAP-25

degradation rates are similar, even though the biological persistence of these toxins is dramatically different. As such, any assertion linking biological persistence to cleaved SNAP-25 removal from the plasma membrane is unwarranted based on the evidence. Furthermore, the fact that SNAP-25 and BoNT/A light chain have substantially different turn-over rates is further evidence that these two proteins are independently removed from the plasma membrane, a finding that is only strengthened by the fact that BoNT/A light chain is present in very limited amounts within the intoxicated cell, whereas SNAP-25 abundant, and rapidly and continuously replaced in the plasma membrane. A person of ordinary skill in the art would not infer that the limited BoNT/A light chain would remained associated with cleaved SNAP-25 as the substrate was removed from the membrane since there would be insufficient light chain to cleave the newly synthesized SNAP-25 that is replacing the cleaved product. However, the fact that the steady-state levels of SNAP-25 replacement and degradation are consistent throughout BoNT/A intoxication indicate that the light chain is continuously cleaving the newly replaced SNAP-25 in the membrane. See, *e.g.*, Keller, 456 (1) FEBS Lett. at 141, col. 2, ¶ 5, line 1 through 142, col. 1, ¶ 1, line 15. Thus the Examiner's contention that BoNT/A association/cleavage of SNAP-25 triggers endocytotsis of the complex is unfounded based on current understanding of membrane removal of cleaved SNAP-25 and BoNT/A light chain and is contrary to what a person of ordinary skill in the art would have understood to be the mechanism of intoxication.

Taken together, a person of ordinary skill in the art knew at the time the present application was filed that 1) BoNT/A is endocytosed when the binding domain from the heavy chain binds to a cell surface receptor and the resulting toxin-receptor complex is endocytosed; 2) SNAP-25 participates in the exocytosis of the neurotransmitter and cleavage prevents this exocytosis; and 3) the BoNT/A light chain and SNAP-25 are not endocytosed together in a complex. As such, the Examiner's assertion that association of a BoNT/A light chain with SNAP-25 triggers endocytosis of the complex is factually incorrect and contrary to the understanding a person of ordinary skill in the art would have with regards to the multi-step intoxication process. Thus, the Examiner has failed to articulate any reasoning with some rational underpinning supporting the assertion that the Fernandez-Salas I abstract taught or suggested the claim limitation.

The Schmidt patent and Fernandez-Salas II abstract are completely silent with respect to the limitations observing the membrane localization pattern of the BoNT/A light chain following

contact of the cell with the test compound and comparing the observed BoNT/A light chain membrane localization pattern to a membrane localization pattern of a BoNT/A light chain in a cell in an absence of the test compound as presently claimed.

Thus, the Examiner has failed to establish by a preponderance of the evidence that the cited prior art has taught each and every limitation of the presently claimed method because the Schmidt patent, the Fernandez-Salas I abstract and Fernandez-Salas II, either alone or in combination, fail to teach or suggest the limitations of observing the membrane localization pattern of the BoNT/A light chain following contact of the cell with the test compound. The mere fact that the Fernandez-Salas I abstract reports that the BoNT/A light chain and SNAP-25 co-localized to the plasma membrane using confocal microscopy would not teach or suggest this claimed limitation. Thus, the Examiner erred, and the Board should therefore reverse the Examiner's 35 U.S.C. § 103(a) obviousness rejections against Claims 1, 3-20, 22, 45-47, 56 and 57.

3) The Examiner erred by failing to establish that the cited prior art taught or suggested each and every element of the presently claimed method.

The Examiner has cited the Schmidt patent in view of the Fernandez-Salas I abstract as the basis of the 35 U.S.C. § 103(a) obviousness rejection. The Examiner has erred in maintaining this rejection because it would not have been obvious to modify or combined the cited prior art to develop the presently claimed methods. This is because the cited art does not teach or suggest a 1) the limitation of a compound that alters biological persistence by observing BoNT/A plasma membrane localization patterns relative to a control as presently claimed; and 2) the limitation of a compound that alters biological persistence by observing BoNT/A plasma membrane localization patterns relative to a control as presently claimed.

Thus, although asserting that the cited references taught or suggested both of these claim limitations, as discussed above, the Examiner's assertion rested on factually incorrect premises and interpretations. The Examiner has failed to submit any evidence or put forth any reasoning based on particular findings of fact demonstrating how a person of ordinary skill in the art would interpret BoNT/A proteolytic activity and BoNT/A biological persistence as defining the same

property. In addition, the Examiner has failed to provide any evidence or articulate reasoning with some rational underpinning indicating how a person of ordinary skill in the art would interpret colocalization of the BoNT/A light chain and SNAP-25 to the plasma membrane as teaching or suggesting the claim limitation of observing the membrane localization pattern of the BoNT/A light chain following contact of the cell with the test compound or comparing the observed BoNT/A light chain membrane localization pattern to a membrane localization pattern of a BoNT/A light chain in a cell in an absence of the test compound. The Examiner has merely relied on broad conclusory statements, personal opinion and mere speculation to assert that the cited prior art has taught each of the elements of the presently claimed method. As such, the Examiner failed to establish by a preponderance of the evidence that the cited art has taught or suggested each and every element of the presently claimed method. Thus, the Examiner erred, and the Board should therefore reverse the Examiner's obvious rejections under 35 U.S.C. § 103(a) against Claims 1, 3-20, 22, 45-47, 56 and 57.

b. The Examiner failed to establish by a preponderance of evidence how the cited art would suggest or motivate a person of ordinary skill in the art to combine the cited art in order to achieve the presently claimed method.

To establish *prima facie* obviousness of a claimed invention, the cited prior art must be considered as a whole and must suggest the desirability and thus the obviousness of making the claimed invention. *MPEP* § 2143; § 2143.02. However, the mere fact that references can be combined or modified does not render the resultant combination obvious unless the references also suggests the desirability of the combination. *Id.* As indicated by the U.S. Supreme Court, "a patent composed of several elements is not proved obvious merely by demonstrating that each of its elements was, independently, known in the prior art." *KSR Int'l Co. v. Teleflex Inc.*, 127 S. Ct. 1727, 1741 (2007). The *KSR* Court affirmed that "rejections on obviousness grounds cannot be sustained by mere conclusory statements; instead, there must be some articulated reasoning with some rational underpinning to support the legal conclusion of obviousness." *Id.* As such, the "factual question of motivation is material to patentability and [can] not be resolved on subjective belief and unknown authority. *Lee*, 277 F.3d at 1344.

The Examiner erred because it would not be obvious for one of ordinary skill in the art to combined the cited art since there was no motivation or suggestion to do so and the nature of the problem to be solved was different.

1) The Examiner erred by failing to established by a preponderance of the evidence that the cited art taught or suggested the presently claimed method of identifying compounds that alter biological persistence biological persistence.

According to MPEP § 2143.01, obviousness can only be established by combining or modifying the teachings of the prior art to produce the claimed invention where there is some teaching, suggestion, or motivation to do so found either explicitly or implicitly in the references themselves or in the knowledge generally available to one of ordinary skill in the art. The Examiner erred to make a *prima facie* obviousness case because the Schmidt patent, the Fernandez-Salas I abstract and Fernandez-Salas II abstract do not provide any motivation, suggestion or teaching that would lead a person skilled in the art to specifically make a method of identifying a compound that either reduces or enhances the biological persistence of BoNT/A as presently claimed.

As discussed above, the presently claimed method comprises a cell-based light-chain localization assay useful for identifying a compound that reduces or enhances a biological persistence of a BoNT/A. *See, supra,* p. 3. The method is based on the ability of a test compound to either shorten the time period that a BoNT/A light chain remains associated with the plasma membrane or lengthen the time period that a BoNT/A light chain remains associated with the plasma membrane. *See, supra,* p. 3. *See, also,* Present specification at ¶¶ 22 and 56 (presently claimed method is referred to as the test localization assay).

The test localization assay does not measure the degree of BoNT/A proteolytic activity that a compound alters at any given time, as does, *e.g.*, the test enzymatic assay disclosed in the present specification. *See, e.g., id.* at ¶¶ 25 and 70. Proteolytic activity measures whether a toxin can cleave a substrate at all and is a completely independent measurement of how long a toxin can persist over time. Thus, a test compound that alters the biological persistence of a BoNT/A light chain and a test compound that alters the proteolytic activity of a BoNT/A light

chain are two separate properties. *See, supra,* pp. 18-19. For example, a test compound may significantly alter the biological persistence of a BoNT/A light chain and yet have no affect on the proteolytic activity of the toxin. Likewise, a test compound may significantly alter the proteolytic activity of a BoNT/A light chain and yet have no affect on the biological persistence of the toxin. The independent and distinct nature of biological persistence and proteolytic activity is also reflected in the present application by the fact that a duel assay that measures biological persistence using the test localization assay and proteolytic activity using the test enzymatic assay is disclosed. *See, e.g., Id.* at ¶¶ 26 and 79.

The Schmidt patent discloses an in vitro substrate-release assay useful for identifying a compound that inhibits or enhances the proteolytic activity of BoNT/A. *See, e.g.*, Schmidt patent at col. 9, line 65 through col. 10, line12; and Claim 9. In this method, a botulinum toxin is "incubated with a test compound . . . transferred to solid supports onto which is immobilized a peptide substrate for the BoNT enzyme being tested . . . and processed." *Id.* at col. 10, lines 1-5. The immobilized peptide substrate is a SNARE peptide that contains a reactive group at its C-terminal end used to attach the substrate to the solid support, a fluorophore group at its N-terminal end used in the fluorescence detection method, and an intervening BoNT cleavage site. *Id.* at col. 7, lines 15-20; and col. 7, lines 26-56. The test sample is mixed with a botulinum toxin and then applied to the matrix. *Id.* at col. 10, lines 1-5. The Schmidt patent indicates that "a reduction in the ability of the toxin to cleave the peptide substrate relative to unincubated toxin indicates a[n] inhibitory compound." *Id.* at col. 10, lines 6-9. On the other hand, "an increase in the ability of the toxin to cleave the peptide substrate relative to unincubated toxin indicated a stimulatory compound." *Id.* at col. 10, lines 9-12. Thus, the Schmidt patent discloses an in vitro substrate-release assay useful for identifying a test compound that inhibits or enhances the proteolytic activity of BoNT/A.

As such, the Schmidt patent is completely silent with respect to any disclosure for 1) any assay that measures biological persistence; 2) a cell-based assay of any sort; 3) a cell-based assay that measures proteolytic activity; 4) a cell-based assay that measures altered biological persistence; 5) a cell-based assay that measures altered biological persistence using light-chain localization as a read-out; let alone 6) a cell-based assay that identifies a compound that reduces or enhances BoNT/A biological persistence using light-chain localization as a read-out.

As such, the Examiner has failed to provide any evidence or articulate any line of reasoning indicating how an in vitro assay using a SNAP-25 substrate merely to detect proteolytic activity would teach or suggest to one of ordinary skill in the art that such findings would also be applicable to a cell-based assay using BoNT/A light plasma membrane localization to detect alterations in biological persistence as presently claimed. Thus, to sustain a *prima facie* case of obviousness the Fernandez-Salas I abstract and/or the Fernandez-Salas II abstract, at a minimum, need to teach, suggest or motivate a person of ordinary skill in the art to fill in these six deficiencies of information in order to achieve the presently claimed methods.

The Fernandez-Salas I abstract discloses that BoNT/A light chains localize in the plasma membrane of neurons in the same compartment as SNAP-25. This abstract also indicates that certain unidentified sequences present in the BoNT/A light chain govern plasma membrane localization. The Fernandez-Salas I abstract speculates that light chain localization may influence the long duration of action of BoNT/A versus BoNT/E at the neuromuscular junction.

The Fernandez-Salas II abstract merely discloses that BoNT/A light chains localize in the plasma membrane of neuronal and non-neuronal cells in the same compartment as SNAP-25. In addition, this abstract suggests that localization of different botulinum light chains play a role in their therapeutic profile and duration of action. As such, this abstract suffers to an even greater extent than the Fernandez-Salas I abstract. However, neither the Fernandez-Salas I abstract nor the Fernandez-Salas II abstract provide any information regarding a cell-based assay useful for identifying a test compound that either reduces or enhances biological persistence of a BoNT/A. 1) a cell-based assay useful for identifying a compound that reduces or enhances BoNT/A biological persistence; let alone 2) a cell-based BoNT/A membrane localization assay useful for identifying a compound that reduces or enhances a biological persistence of a BoNT/A.

Thus, the Examiner has failed to provide any evidence or articulate any line of reasoning indicating how the finding that light chain localization may influence biological persistence would teach or suggest to one of ordinary skill in the art that such findings would also be applicable to a cell-based assay to screen for other compounds that alter BoNT/A biological persistence using BoNT/A light plasma membrane localization to detect alterations in biological persistence

as presently claimed. Thus, the Fernandez-Salas I abstract and/or the Fernandez-Salas II abstract does not teach, suggest or motivate a person of ordinary skill in the art to develop a cell-based assay that identifies a compound that reduces or enhances BoNT/A biological persistence using light-chain localization as a read-out. Thus, neither abstract, either alone or with the other abstract, overcome the deficiencies of the Schmidt patent and a *prima facie* case of obviousness cannot be sustained.

The Examiner contends that a person of ordinary skill in the art would have been motivated to combine the Schmidt patent and the Fernandez-Salas I abstract "to screen a compound that affects a biological persistence of a Clostridial toxin by evaluating the localization and enzymatic activity." October 3, 2006 Office Action at p. 13, ¶ 1, lines 1-3. The Examiner supports this contention on the ground that "[t]he biological persistence of proteolytic activity of BoNT/A relies on the proteolytic activity of the light chain. In addition, the interaction of BoNT/A with its substrate, such as SNAP-25, occurs intracellularly because the interaction of BoNT/A triggers the internalization of the complex and subsequently cleaves SNAP-25 and inhibits exocytosis of synaptic vesicles." *Id.* at lines 3-8. However, the assertions cited by the Examiner are without merit and run contrary to the knowledge a person of ordinary skill in the art knew at the time the present application was filed.

First, the Examiner is factually incorrect in asserting that BoNT/A biological persistence relies on the proteolytic activity of the light chain. As discussed above, the present specification clearly indicates that biological persistence and proteolytic activity are distinct and independent characteristics of BoNT/A functionality and each characteristic can be assayed separately using the methods disclosed in the present specification. *See, supra,* pp. 18-19.

Second, the Examiner statement that "the interaction of BoNT/A with its substrate, such as SNAP-25, occurs intracellularly because the interaction of BoNT/A triggers the internalization of the complex and subsequently cleaves SNAP-25 and inhibits exocytosis of synaptic vesicles" is irrelevant. The Examiner has merely summarized the multi-step intoxication process discussed above. *See, supra,* p. 2. However, the Examiner has failed to articulate a compelling line of reasoning why the knowledge of this intoxication process would provide any suggestion to a person of ordinary skill in the art to develop any screening method for biological persistence in

view of the cited art, let alone the presently claimed method of a cell-based assay that identifies a compound that reduces or enhances BoNT/A biological persistence using BoNT/A light-chain localization as a read-out. Specificity is a requirement because "particular findings must be made as to the reason the skilled artisian, with no knowledge of the claimed invention, would have selected these components for combination in the manner claimed." *In re Kotzab*, 217 F.3d at 1365, 1371. Thus, the Examiner's assertion that BoNT/A interaction with SNAP-25 occurs intracellularly because the interaction of BoNT/A triggers the internalization of the complex and subsequently cleaves SNAP-25 and inhibits exocytosis of synaptic vesicles is a general restatement of the intoxication process, and not particular findings of fact as to the reason why a person of ordinary skill in the art, with no knowledge of the claimed method, would have combined the disclosure form the cited art to achieve the presently claimed method. As such, the Examiner has merely voiced personal opinion and mere speculation to support this assertion.

Thus, the Examiner has failed to established by a preponderance of the evidence that the cited art, either alone or in combination, provide any teaching, suggestion or motivation to develop 1) a cell-based assay useful for identifying a compound that reduces or enhances a biological persistence of a BoNT/A; let alone 2) a cell-based BoNT/A membrane localization assay useful for identifying a compound that reduces or enhances a biological persistence of a BoNT/A, in order to arrive at the presently claimed invention. Thus, the Examiner erred, and the Board should therefore reverse the Examiner's obvious rejections under 35 U.S.C. § 103(a) against Claims 1, 3-20, 22, 45-47, 56 and 57.

2) The Examiner erred since the Schmidt patent, Fernandez-Salas I abstract and Fernandez-Salas II abstract combination makes the Schmidt patent unsatisfactory for its intended use.

According to MPEP §2143.01, if proposed modification would render the prior art invention being modified unsatisfactory for its intended purpose, then there is no suggestion or motivation to make the proposed modification. The Examiner erred because combining the Schmidt patent with the Fernandez-Salas I abstract and Fernandez-Salas II abstract, as suggested by the Examiner, would render the substrate-release assay disclosed in the Schmidt patent inoperable

for its intended use since such a combination would prevent the ability of the assay to determine proteolytic activity.

The presently claimed methods are directed toward a cell-based light-chain localization assay useful for identifying a compound that either reduces or increases a biological persistence of a BoNT/A. *See, supra,* p. 3; and *infra,* p. 45, Claim 1. In this method, a test compound is incubated with a cell containing a BoNT/A light chain localized to the cytoplasmic side of the plasma membrane and changes in the intracellular localization pattern of the toxin over time are determined. *Id.* The BoNT/A light chain can be expressed by the cell from an expression construct or transfected into the cell, see, *e.g.*, ¶¶ 63-67. If the BoNT/A light chain remains associated with the plasma membrane for a longer period of time in a cell contacted with the test compound relative to the length of time the light chain remains associated with the membrane in a cell not contacted with the test compound (a control cell), then the test compound is deemed to increase biological persistence. If the BoNT/A light chain remains associated with the plasma membrane for a shorter period of time in a cell contacted with the test compound relative to the length of time the light chain remains associated with the membrane in a control cell, then the test compound is deemed to decrease biological persistence. Note, that the key read-out of the presently claimed method is light chain localization. A BoNT/A light chain can be disassociated from the plasma membrane and still be proteolytically active. Thus, a test compound could be categorized as reducing BoNT/A biological persistence because it mediates a faster disassociation of the light chain from the membrane relative to a control, yet that disassociated light chain can still have full proteolytic activity. This is because biological persistence and proteolytic activity are distinct and separate properties.

The Schmidt patent discloses an in vitro substrate-release assay useful for identifying a compound that inhibits or enhances the proteolytic activity of BoNT/A. *See, supra,* p. 30. In this method, a solid support is made that contains a SNAP-25 fragment that serves as the substrate for a BoNT/A. A BoNT/A is incubated with a test compound in solution and this solution is then applied to the solid support containing the SNAP-25 substrate. As the BoNT/A comes in contact with its immobilized SNAP-25 substrate, the toxin cleaves it, whereby the cleaved SNAP-25 product is released from the solid support and collected for subsequent detection analysis. To

test for the effects of a test compound of BoNT/A proteolytic activity, a test compound is mixed with BoNT/A and then applied to this matrix. *Id.* If more SNAP-25 product is eluted from the column relative to a BoNT/A sample not containing the test compound (a control sample), then the test compound is deemed to increase proteolytic activity of BoNT/A. *Id.* If less SNAP-25 product is eluted from the column relative to a control sample, then the test compound is deemed to decrease proteolytic activity of BoNT/A. *Id.* As such, the ability of a test compound to alter the amount of proteolytic cleavage is the basis for determining whether a test compound inhibits BoNT activity (*i.e.*, reduced proteolytic cleavage relative to a control) or enhances proteolytic cleavage (*i.e.*, increases proteolytic cleavage relative to a control).

Replacing the immobilized SNARE substrate with a BoNT/A light chain on the solid support, as suggested by the Examiner, would result in an inoperable substrate-release assay as disclosed in the Schmidt patent. The Schmidt patent method relies on the ability of the BoNT to cleave the SNARE substrate immobilized on the solid support. If there is no SNARE protein, there is no substrate to be cleaved by the toxin, and thus no way of determining to what extent a test compound decreases or increases the amount of substrate proteolytically cleaved by a botulinum toxin light chain. Additionally, adding a BoNT/A light chain to the solid support, so that both the SNARE substrate and the BoNT/A light chain are present on the solid support, is also inoperable. The presence of the BoNT/A light chain on the support would result in the SNARE substrate being continuously cleaved by the toxin. As such, the influence that a test compound may have on a Clostridial toxin's proteolytic activity will be completely masked because the assay will always indicate that the applied toxin has full proteolytic activity.

In addition, modification of the in vitro substrate-release assay disclosed in the Schmidt patent into a cell-based assay will also result in an inoperable substrate-release assay. This is because the substrates disclosed in this patent lack the required membrane targeting sequences and are thus incapable of localizing to the plasma membrane. For example, studies suggest two conserved motifs present within the interhelical loop region of SNAP-25 are responsible for membrane targeting. See, *e.g.*, Susana Gonzalo et al., *SNAP-25 is Targeted to the Plasma Membrane Through a Novel Membrane-Binding Domain*, 274(30) J. Biol. Chem. 21313-21318 (1999) at 21317, col. 1, ¶ 2, line 1 through col. 2, ¶ 1, line 10. One of these motifs is a cysteine-rich region comprising amino acids 84-92 of full-length SNAP-25. *Id.* at 21317, col.

1, ¶ 1, lines 5-6. Palmitoylation of the cysteines comprising this region are necessary for membrane localization of SNAP-25 because elimination of these cysteine residues results in a loss of SNAP-25 membrane-targeting. *See, e.g.,* Michael Veit et al., *Multiple Palmitoylation of Synaptotagmin and the t-SNARE SNAP-25*, 385(1-2) FEBS Lett. 119-123 (1996) at 121, col. 2, ¶ 1, line 1 through 122, col. 1, ¶ 2, line 16 and 122, col. 2, ¶ 2, lines 1-8; and Stacie R. Lane and Yuechueng Liu, *Characterization of the Palmitoylation Domain of SNAP-25*, 69(5) J. Neurochem. 1864-1869 (1997) at 1867, col. 2, ¶ 2, lines 1-20. Thus, the SNAP-25 substrates disclosed in the Schmidt patent cannot localize to the plasma membrane because these substrates lack the cysteine-rich region comprising amino acids 84-92. As such, incorporation of the SNAP-25 substrates of this patent into a cell, as suggested by the Examiner, would result in an inoperable assay because the BoNT/A light chain would be unable to cleave its substrate since it would never co-localize with the light chain. Since the BoNT/A light chain cannot cleave its substrates, the substrate-release assay would be incapable of detecting BoNT/A proteolytic activity and compounds that would increase or decrease this activity, thereby making disclosed in the Schmidt patent inoperable for its indented use.

Neither the Fernandez-Salas I abstract and Fernandez-Salas II abstract provide any disclosure contrary to the teachings of the Schmidt patent or the art. As such, these abstracts cannot remedy the inoperable substrate-release assay resulting from the Examiner's suggested combination.

Thus, replacement of a SNARE substrate with a BoNT/A light chain would make the substrate-release assay disclosed in the Schmidt patent inoperable for its indented use. In addition, integration of the SNAP-25 substrates disclosed in the Schmidt patent into a cell will likewise result in an inoperable substrate-release assay. As such, the Examiner erred in considering the Schmidt patent in its entirety and has failed to make a *prima facie* case of obviousness because combining the Schmidt patent with the Fernandez-Salas I abstract and Fernandez-Salas II abstract as suggested by the Examiner would result in an inoperable substrate-release assay. Thus, the Examiner erred, and the Board should therefore reverse the Examiner's obvious rejections under 35 U.S.C. § 103(a) against Claims 1, 3-20, 22, 45-47, 56 and 57.

3) The Examiner erred since the Schmidt patent, Fernandez-Salas I abstract and Fernandez-Salas II abstract combination changes the principle of operation of the Schmidt patent.

According to MPEP §2143.01, if a proposed modification or combination of the prior art would change the principle of operation of the prior art invention being modified, then the teachings of the references are not sufficient to render the claims *prima facie* obvious. The Applicants respectfully submit that it would not be obvious for one of ordinary skill in the art to combine the cited references because combining the Schmidt patent with the Fernandez-Salas I abstract and Fernandez-Salas II abstract would change the principle of operation of the substrate-release assay disclosed in the Schmidt patent.

As discussed above, the presently claimed method comprises a cell-based light-chain localization assay useful for identifying a compound that reduces or enhances a biological persistence of a BoNT/A.

As discussed above, the Schmidt patent discloses an in vitro substrate-release method useful for identifying a compound that inhibits or enhances the proteolytic activity of BoNT/A. The ability of a test compound to alter the amount of proteolytic cleavage is the basis for determining whether a test compound inhibits BoNT activity (*i.e.*, reduced proteolytic cleavage relative to a control) or enhances proteolytic cleavage (*i.e.*, increases proteolytic cleavage relative to a control).

As discussed above, both the Fernandez-Salas I abstract and the Fernandez-Salas II abstract discloses that BoNT/A light chains localize in the plasma membrane of neuronal and non-neuronal cells in the same compartment as SNAP-25.

The suggested combining of the Schmidt patent with the Fernandez-Salas I abstract and Fernandez-Salas II abstract would change the principle of operation of the assays disclosed in the Schmidt patent in at least two ways.

First, the molecule used as the read-out of a test compound's activity between the Schmidt patent and the present claimed methods operates under a different principle. The Schmidt patent discloses a substrate-release assay that uses a SNAP-25 substrate which serves as a read-out for the proteolytic activity of the BoNT, referred to as a Type (II) Substrate, see, col. 7, line 14 through col. 9, line 34. As such, the principle of operation is to determine to what extent a test compound can decrease or increase the amount of SNARE substrate proteolytically cleaved by a botulinum toxin light chain. The Schmidt patent also discloses FRET-based substrates used in a quench-release assay. See, Schmidt patent at col. 5, line 8 through col. 7, line 12. On the other hand, the presently claimed methods are directed toward a toxin localization assay that uses the length of time that a BoNT/A light chain remains associated with the plasma membrane as a read-out for biological persistence of the BoNT. As such, the principle of operation of the read-out is to determine to what extent a test compound decreases or increases the length of time a BoNT/A light chain remains associated with the plasma membrane. Thus, the principle of operation between these two methods are different because the Schmidt patent assay relies on the level of enzymatic proteolysis of a substrate, whereas, the presently claimed method relies on the length of time that a light chain remains associated with a membrane.

Second, the mechanics of the assay between the Schmidt patent and the present claimed methods operates under a different principle. The Schmidt patent discloses an in vitro substrate release assay where the SNARE substrate is immobilized on a solid support, a solution containing a BoNT and a test compound is poured over this solid support, the flow-through is collected and then tested for fluorescence. *Id.* at col. 4, lines 57-67; and col. 8, line 57 through col. 9, line 16. The SNARE substrates contain a reactive group at its C-terminal end used to attach the substrate to the solid support, a fluorophore group at its N-terminal end used in the fluorescence detection method, and an intervening BoNT cleavage site. *Id.* at col. 7, lines 15-20; and col. 7, lines 26-56. For this assay to work, the cleavage product must be removed from the solid support matrix and transferred to a separate container. *Id.* at col. 4, lines 23-30. This is because both cleaved products and uncleaved SNAP-25 substrates contain the fluorophore and if they remain together in would be impossible to distinguished the substrates from the products using a fluorimeter. As such, the assay cannot be performed in a cell because there would be no way to separate the cleaved product from the uncleaved SNAP-25 substrate.

Thus, the principle of operation of the assay relies on an in vitro-based, solid support matrix that enables the separation of the cleaved product from the uncleaved SNAP-25 substrate. On the other hand, the presently claimed method is a cell-based toxin localization assay where a cell containing a BoNT/A light chain is exposed to a test compound and the localization pattern of the BoNT/A light chain is determined over time. This method does not require the separation of the BoNT/A light chain from the cell. Additionally, this method requires a cell and cannot be performed as an in vitro assay because of the essential requirement that a BoNT/A light chain be associated with the plasma membrane. Thus, the principle of operation between these two methods are different because the Schmidt patent assay relies on the in vitro assay that requires the separation of cleavage products from the uncleaved substrates, whereas, the presently claimed method relies on a cell-based assay that requires a differential localization of a toxin over time.

Thus, replacing the SNAP-25 substrate with the BoNT/A light chain would change the principle of operation from an in vitro enzymatic-based assay that uses changes in substrate cleavage amounts as the principle of operation to a cell-based light chain localization assay that uses changes in the membrane localization pattern of a protein as the principle of operation. In order for the substrate-release assay disclosed in the Schmidt patent to incorporate the suggested modifications, the substrate-release assay would have to change 1) its read-out from one that relies on the level of enzymatic proteolysis of a substrate to one that relies on the length of time that a toxin remains associated with a membrane; 2) its mechanics from one that relies on an in vitro based assay that requires separation of a product from its substrate to one that relies on a cell-based assay that requires differential patterns of membrane localization of a toxin over time. However, neither the Fernandez-Salas I abstract and Fernandez-Salas II abstract provide any disclosure teaching or suggesting these changes in operation. As such, this abstracts cannot remedy the change in the principle of operation resulting from the Examiner's suggested combination.

Therefore, the Examiner failed to make a *prima facie* case of obviousness because the combining the Schmidt patent with the Fernandez-Salas I abstract and Fernandez-Salas II abstract, as suggested by the Examiner, would change the principle of operation of the substrate-release assay disclosed in the Schmidt patent. Thus, the Examiner erred, and the

Board should therefore reverse the Examiner's obvious rejections under 35 U.S.C. § 103(a) against Claims 1, 3-20, 22, 45-47, 56 and 57.

4) The Examiner erred by failing to consider that the nature of the problem solved in the cited prior art was different from the problem solved in the presently claimed method.

According to *MPEP* § 2143.01, the nature of the problem to be solved as a whole may be used as evidence to establish a motivation to combine the cited art. As such, the Examiner erred in combining the cited prior art because the nature of the problems solved by the Schmidt patent, the Fernandez-Salas I abstract and Fernandez-Salas II abstract were very different from the nature of the problem solved by the presently claimed method.

The problem identified in the present application is the lack of a screening assay that can detect compounds that alter biological persistence of a botulinum toxin. The solution is, in part, the claimed method which utilizes the subcellular localization of the BoNT/A light chain to the plasma membrane. The length of time that a BoNT/A light chain remains associated with the plasma membrane is directly correlated to the biological persistence of the toxin. Utilizing this finding, the presently claimed method screens for test compounds which alter the duration that a BoNT/A light chain remains associated with the plasma membrane. A test compound that increases the length of time that the light chain remains associated with the plasma membrane is indicative of a compound that increases the biological persistence of BoNT/A. A test compound that decreases the length of time that the light chain remains associated with the plasma membrane is indicative of a compound that reduces the biological persistence of BoNT/A. Thus, the problem identified in the present application was the need for a screening method that could identify compounds that alter botulinum toxin biological persistence and the solution was a method that determined whether a compound altered the length of time a BoNT/A light chain remained associated with the plasma membrane.

The problem identified in the Schmidt patent was the need for a in vitro proteolytic activity assay for botulinum toxins that overcame the known drawbacks of previous method that required significantly more time, were more complex due to extra steps, and were more expensive to

perform. Schmidt patent at col. 3, lines 27-29. *See, also, Id.* at col. 2, line 30 through col. 3, line 26. The solution, in part, was to develop fluorescence-based substrates that overcame or eliminated these drawbacks. *Id.* at col. 4, lines 14-36. The disclosed substrates were categorized into two types. The first type were FRET-based SNARE substrates of approximately 17-39 amino acids in length useful in quench-release assays. *Id.* at col. 5, line 8 through col. 7, line12. The second type were fluorescence-based SNARE substrates of approximately 24-116 amino acids in length useful in substrate-release assays. *Id.* col. 7, line 13 through col. 9, line 34. Thus, the problem identified in the Schmidt patent was the need for a better proteolytic activity assay for botulinum toxins and the solution was the development of fluorescence-based substrates that overcame many of the problems of previous proteolytic activity assays.

The problem identified in the Fernandez-Salas I abstract was the need for data to explain the basis for the long duration of BoNT/A light chain activity. Fernandez-Salas I abstract at lines 8-9. The solution was to examine subcellular localization of fluorescently-labeled light chains of BoNT/A and BoNT/E in order to determine any apparent differences. *Id.* at 9-19. GFP-labeled BoNT/A light chain and GFP-labeled-BoNT/E light chains were separately transformed into a neurons and the subcellular localization of these labeled light chains was determine by confocal microscopy. *Id.* Thus, the problem was a lack of understanding for the basic molecular mechanisms governing biological persistence and the solution was to examine the subcellular localization of fluorescently-labeled light chains of BoNT/A and BoNT/E.

The problem identified in the Fernandez-Salas II abstract was the need for data to explain the basis for the long duration of BoNT/A light chain activity. Fernandez-Salas II abstract at lines 6-7. The solution was to examine subcellular localization of fluorescently-labeled light chains of BoNT/A, BoNT/B and BoNT/E in order to determine any apparent differences. *Id.* at 7-24. GFP-labeled BoNT/A light chain, GFP-labeled BoNT/B light chain and GFP-labeled-BoNT/A light chains were separately transformed into both neuronal and non-neuronal cells and the subcellular localization of these labeled light chains was determine by confocal microscopy. *Id.* Thus, the problem was a lack of understanding for the basic molecular mechanisms governing biological persistence and the solution was to examine the subcellular localization of fluorescently-labeled light chains of BoNT/A, BoNT/B and BoNT/E.

As such, the nature of the problems solved in the cited prior art are unrelated to the problem addressed by the presently claimed method of screening for compounds that alter biological persistence of a botulinum toxin. For example, the Schmidt patent was addressing the need to improve in vitro proteolytic activity assays for botulinum toxins. Both the Fernandez-Salas I abstract and he Fernandez-Salas II abstract were concerned with identifying the underlying mechanisms that confer a longer biological persistence to BoNT/A relative to other botulinum toxins. Although both abstracts discloses that plasma membrane localization of the BoNT/A light chain played a role in biological persistence, such observations do not teach or suggest the development of a screening assay to identify compounds that alter BoNT/A biological persistence.

Although the Examiner contends that proteolytic activity and biological persistence are one in the same property, this claim is unsubstantiated by the evidence of record. The cited art makes it clear that such equation is groundless. For example, the Fernandez-Salas II abstract investigated biological persistence using fluorescently-labeled light chains and confocal microscopy. However, proteolytic activity was determined using western blot analysis. *Id.* at 10-11. If proteolytic activity and biological persistence were one in the same property, then why two distinct assays? The Examiner has failed to provide any evidence or reasoning based on particular findings of fact that would teach or suggest that a person of ordinary skill in the art would combine the cited references in order to achieve the presently claimed method. As such, the Examiner has failed to consider the cited prior art in its entirety because the Examiner has failed to considered that the teachings of the cited art were directed towards solving a problem different from the one solved by the Applicant's presently claimed toxins. Thus, the Examiner erred, and the Board should therefore reverse the Examiner's obvious rejections under 35 U.S.C. § 103(a) against Claims 1, 3-20, 22, 45-47, 56 and 57.

c. The Examiner failed to establish by a preponderance of evidence that there was a reasonable expectation of success for making the proposed modification or combination.

The Examiner asserts that a person of ordinary skill in the art would have expected "success in screening a compound that reduces/increases a biological persistence of a BoNT/A by contacting cells expressing a light chain of BoNT/A with test compounds and evaluating the localization and proteolytic activity of the BoNT/A light chain on SNAP-25" October 3, 2006 Office Action at p. 13, ¶ 1, lines 8-12. The Examiner supports this assertion on the basis that "[t]he biological persistence of proteolytic activity of BoNT/A relies on the proteolytic activity of the light chain. In addition, the interaction of BoNT/A with its substrate, such as SNAP-25, occurs intracellularly because the interaction of BoNT/A triggers the internalization of the complex and subsequently cleaves SNAP-25 and inhibits exocytosis of synaptic vesicles." *Id.* at lines 3-8.

According to *MPEP* § 2143.02 a case for *prima facie* obviousness requires that a reasonable expectation of success be suggested or expressed in the prior art combination, and that "[e]vidence showing there was no reasonable expectation of success may support a conclusion of nonobviousness." See, also, *MPEP* § 2143. The Examiner erred because it would not be obvious to combine the cited references since a person of ordinary skill in the art would not have had a reasonable expectation that the proposed modification or combination would be successful.

In support of the Examiner's contention that a person of ordinary skill in the art would have a reasonable expectation of success for making the proposed combination, the Examiner first asserts that BoNT/A biological persistence relies on the proteolytic activity of the light chain. However, as discussed above, this assertion is incorrect because the property of biological persistence does not rely on proteolytic activity of the toxin, but on light chain localization to the plasma membrane. *See, supra,* pp. 18-19.

The Examiner also asserts as evidence for a reasonable expectation of success the comment that BoNT/A interaction with SNAP-25, occurs intracellularly because the interaction of BoNT/A triggers the internalization of the complex and subsequently cleaves SNAP-25 and inhibits exocytosis of synaptic vesicles. However, the Examiner has failed to explain how the mere fact that cleavage of SNAP-25 occurs intracellularly provides evidence of a reasonable expectation of success that a test compound that alters BoNT/A persistence can be identified by

determining whether that compound alters the length of time the light chain remains associated with the plasma membrane. As discussed above, this statement by the examiner has merely reiterated the multi-step intoxication process. *See, supra,* p. 2; and pp. 32-33.

Therefore, the Examiner's assertion that the cited prior art provides a reasonable expectation of success is without basis because the Examiner has failed to establish a *prima facie* case by a preponderance of the evidence. In addition, the teachings of the cited prior art would not provide a reasonable expectation of success that the proposed modification would produce the presently claimed method of identifying compounds that increase or decrease biological persistence. Thus, the Examiner erred, and the Board should therefore reverse the Examiner's obvious rejections under 35 U.S.C. § 103(a) against Claims 1, 3-20, 22, 45-47, 56 and 57.

2. *The Examiner failed to establish any of the elements necessary to make a prima facie case of obvious against* Claims 1, 3-20, 22, 45-47, 56 and 57*.*

For all the reasons stated above, the Examiner has failed to establish by a preponderance of evidence each element required to make a *prima facie* case of obviousness. Therefore, the Examiner has erred, and the Board therefore reverse the Examiner's obvious rejections under 35 U.S.C. § 103(a) against Claims 1, 3-20, 22, 45-47, 56 and 57.

VIII. CLAIMS APPENDIX

The following claims are at issue in this appeal.

1. (Previously presented) A method of identifying a compound that either reduces or increases a biological persistence of a BoNT/A, the method comprising a test localization assay comprising the steps of:

   (a) contacting a cell that comprises a BoNT/A light chain with a test compound, wherein the BoNT/A light chain displays an intracellular localization pattern at the plasma membrane;

   (b) observing the membrane localization pattern of the BoNT/A light chain following contact of the cell with the test compound; and

   (c) comparing the observed BoNT/A light chain membrane localization pattern to a membrane localization pattern of a BoNT/A light chain in a cell in an absence of the test compound;

   wherein a reduced membrane localization pattern of the BoNT/A light chain over time in the cell contacted with the test compound as compared to the membrane localization pattern of the BoNT/A light chain over time in the cell in the absence of the test compound is indicative of a test compound that reduces the biological persistence of a BoNT/A; and

   wherein an increased membrane localization pattern of the BoNT/A light chain over time in the cell contacted with the test compound as compared to the membrane localization pattern of the BoNT/A light chain over time in the cell in the absence of the test compound is indicative of a test compound that increases the biological persistence of a BoNT/A.

2. (Cancelled)

3. (Previously presented) The method of claim 1 wherein in step (c) an observed increased biological persistence is about 20% to about 300% more BoNT/A light chain localized to the plasma membrane over time in the cell contacted with the test compound as compared to the BoNT/A light chain localized to the plasma membrane over time in the cell in the absence of the test compound, said more membrane localization pattern being indicative of a test compound that increases the biological persistence of a BoNT/A.

4. (Previously presented) The method of claim 1 wherein in step (c) an observed reduced biological persistence is about 10% to about 90% reduction in plasma membrane localization of the BoNT/A light chain over time in the cell contacted with the test compound as compared to the membrane localization of the BoNT/A light chain over time in the cell in the absence of the test compound, said reduced membrane localization pattern being indicative of a test compound that reduces the biological persistence of a BoNT/A.

5. (Previously presented) The method of claim 1 further comprising a negative control localization assay comprising the steps of:

   (a) contacting a cell that comprises the BoNT/A light chain with a localization assay negative control compound, wherein the localization assay negative control compound is a compound known to have no effect on the membrane localization pattern of the BoNT/A light chain in a cell; and

   (b) determining whether the membrane localization pattern of the BoNT/A light chain in the cell differs following contacting the cell with the localization assay negative control compound compared to the membrane localization pattern of the BoNT/A light chain in the cell in the absence of the localization assay negative control compound,

wherein a change in the membrane localization pattern of the BoNT/A light chain in the cell following contacting the cell with the localization assay negative control compound indicates that the test localization assay results are inconclusive.

6. (Previously presented) The method of claim 1 further comprising a positive control localization assay comprising the steps of:

(a) contacting a cell that comprises the BoNT/A light chain with a localization assay positive control compound, wherein the localization assay positive control compound is a compound known to change the membrane localization pattern of the BoNT/A light chain in a cell; and

(b) determining whether the membrane localization pattern of the BoNT/A light chain in the cell differs following contacting the cell with the localization assay positive control compound compared to the membrane localization pattern of the BoNT/A light chain in the cell in the absence of the localization assay positive control compound,

wherein an absence of change in the localization pattern of the light chain in the cell following contacting the cell with the localization assay positive control compound indicates that the test localization assay results are inconclusive.

7. (Original) The method of claim 1 comprising multiple test localization assays wherein individual test assays are performed using different concentrations of test compound.

8. (Original) The method of claim 1 comprising performing at least a duplicate test localization assays.

9. (Original) The method of claim 1 wherein the cell is selected from the group consisting of: Neuro-2A cells, PC12 cells, SHSY-5Y cells, HIT-T15 cells, HeLa cells, HEK293 cells, and primary and established neuronal culture cells from spinal cord, cortex, hippocampus and dorsal root ganglion.

10. (Previously presented) The method of claim 1 wherein the cell comprises a gene that encodes the BoNT/A light chain, which is expressed to produce the BoNT/A light chain in the cell.

11. (Previously presented) The method of claim 1 wherein a BoNT/A is contacted with the cell in an amount effective to be taken up by the cell, the amount effective to be taken up by the cell being the amount able to produce an identifiable membrane localization pattern of the BoNT/A light chain in the cell.

12. (Previously presented) The method of claim 1 wherein the BoNT/A light chain is labeled.

13. (Previously presented) The method of claim 1 claim 12 wherein the labeled BoNT/A light chain is labeled with a radio-active isotope or a fluorescent marker.

14. (Previously presented) The method of claim 1 wherein the BoNT/A light chain is expressed as a fusion protein comprising a BoNT/A light chain fused with a fluorescent marker.

15. (Previously presented) The method of claim 1 wherein the membrane localization pattern is determined using microscopic techniques that allow for the analysis of changes in subcellular localization, including confocal microscopic systems.

16. (Previously presented) The method of claim 1 further comprising a test enzymatic assay comprising the steps of:

(a) contacting a sample containing the BoNT/A light chain with a SNAP-25 substrate in the presence of the test compound; and

(b) determining whether the SNAP-25 substrate is processed by the BoNT/A light chain into enzymatic product;

wherein the absence of processing of the SNAP-25 substrate into enzymatic product indicates that the test compound inhibits BoNT/A enzymatic activity, and the enhancement of processing of the SNAP-25 substrate into enzymatic product indicates that the test compound enhances BoNT/A enzymatic activity.

17. (Previously presented) The method of claim 16 further comprising a negative control enzymatic assay comprising the steps of:

(a) contacting a sample that comprises the BoNT/A light chain with a SNAP-25 substrate in the presence of an enzymatic assay negative control compound or no added compound, wherein the enzymatic assay negative control compound is a compound known not to inhibit BoNT/A enzymatic activity; and

(b) determining whether the SNAP-25 substrate is processed by the BoNT/A light chain into enzymatic product;

wherein the absence of processing of the SNAP-25 substrate into enzymatic product indicates that test enzymatic assay results are inconclusive.

18. (Previously presented) The method of claim 16 further comprising a positive control enzymatic assay comprising the steps of:

(a) contacting a sample that comprises the light chain with a SNAP-25 substrate in the presence of an enzymatic assay positive control compound, wherein the enzymatic assay positive control compound is a compound known to inhibit BoNT/A enzymatic activity; and

(b) determining whether the SNAP-25 substrate is processed by the BoNT/A light chain into enzymatic product;

wherein processing of the SNAP-25 substrate into enzymatic product indicates that test enzymatic assay results are inconclusive.

19. (Original) The method of claim 16 comprising multiple test enzymatic assays wherein individual enzymatic test assays are performed using different concentrations of test compound.

20. (Original) The method of claim 16 comprising performing at least duplicate test enzymatic assays.

21. (Cancelled)

22. (Previously presented) The method of claim 16 wherein the processing of it into enzymatic product is determined by Western blot, ELISA assay, GFP-SNAP assay, FRET assay, or a combination of said assays, using an antibody that specifically binds to uncleaved SNAP-25 substrate and/or enzymatic products.

23-44. (Cancelled)

45. (Previously presented) A method of identifying a compound that either reduces or increases a biological persistence of a BoNT/A, the method comprising the steps of:

(a) contacting a cell that comprises a BoNT/A light chain with a test compound, wherein the BoNT/A light chain displays an intracellular localization pattern at the plasma membrane; and

(b) determining whether the membrane localization pattern of the BoNT/A light chain is reduced or increased in the cell contacted with the test compound as compared to the membrane localization pattern of the BoNT/A light chain in a cell in the absence of the test compound,

wherein a reduced membrane localization pattern of the BoNT/A light chain over time in the cell contacted with the test compound as compared to the membrane localization pattern of the BoNT/A light chain over time in the cell in the absence of the test

compound is indicative of a test compound that reduces the biological persistence of a BoNT/A; and

wherein an increased membrane localization pattern of the BoNT/A light chain over time in the cell contacted with the test compound as compared to the membrane localization pattern of the BoNT/A light chain over time in the cell in the absence of the test compound is indicative of a test compound that increases the biological persistence of a BoNT/A.

46. (Previously presented) The method of claim 45 wherein in step (b) a determined increased biological persistence is about 20% to about 300% more BoNT/A light chain localized to the plasma membrane over time in the cell contacted with the test compound as compared to the BoNT/A light chain localized to the plasma membrane over time in the cell in the absence of the test compound, said more membrane localization pattern being indicative of a test compound that increases the biological persistence of a BoNT/A.

47. (Previously presented) The method of claim 45 wherein in step (b) a determined reduced biological persistence is about 10% to about 90% reduction in plasma membrane localization of the BoNT/A light chain over time in the cell contacted with the test compound as compared to the membrane localization of the BoNT/A light chain over time in the cell in the absence of the test compound, said reduced membrane localization pattern being indicative of a test compound that reduces the biological persistence of a BoNT/A.

48-55 (Cancelled).

56. (Previously presented) The method of claim 1 wherein in step (c) an observed reduced biological persistence is a more than 20% reduction in BoNT/A light chain density at the plasma membrane over time in the cell contacted with the test compound as compared to the BoNT/A light chain density at the plasma membrane over time in the cell in the

absence of the test compound, said reduced BoNT/A light chain density being indicative of a test compound that decreases the biological persistence of a BoNT/A.

57. (Previously presented) The method of claim 45 wherein in step (b) a determined reduced biological persistence is a more than 20% reduction in BoNT/A light chain density at the plasma membrane over time in the cell contacted with the test compound as compared to the BoNT/A light chain density at the plasma membrane over time in the cell in the absence of the test compound, said reduced BoNT/A light chain density being indicative of a test compound that decreases the biological persistence of a BoNT/A.

IX. EVIDENCE APPENDIX

Michael Adler et al., *Persistence of Botulinum Neurotoxin A Demonstrated by Sequential Administration of Serotypes A and E in Rat EDL Muscle*, 39 Toxicon 233-243 (2001).

Jeffery M. Besterman and Robert B. Low, *Endocytosis: A Review of Mechanisms and Plasma Membrane Dynamics*, 210 Biochem. J. 1-13 (1983).

Patrick G. Foran et al., *Evaluation of the Therapeutic Usefulness of Botulinum Neurotoxin B, C1, E, and F Compared with the Long Lasting Type A*, 278(2) J. Biol. Chem. 1363-1371 (2003).

Susana Gonzalo et al., *SNAP-25 is Targeted to the Plasma Membrane Through a Novel Membrane-Binding Domain*, 274(30) J. Biol. Chem. 21313-21318 (1999).

James E. Keller et al., *Persistence of Botulinum Neurotoxin Action in Cultured Spinal Cord Cells*, 456 (1) FEBS Lett. 137-142 (1999).

D. Borden Lacy et al., *Crystal Structure of Botulinum Neurotoxin Type A and Implications for Toxicity*, 5(10) Nature Struct. Biol. 898-902 (1998).

D. Borden Lacy and Raymond C. Stevens, *Sequence Homology and Structural Analysis of the Clostridial Neurotoxins*, 291(5) J. Mol. Biol. 1091-11-4 (1999).

Stacie R. Lane and Yuechueng Liu, *Characterization of the Palmitoylation Domain of SNAP-25*, 69(5) J. Neurochem. 1864-1869 (1997).

Arleen Loewy et al., *The Major ^{35}S-Methionine-Labeled Rapidly Transported Protein (Superprotein) is Identical to SNAP-25, a Protein of Synaptic Terminals*, 11(11) J. Neurosci. 3412-3421 (1991).

Cesare Montecucco and Giampierto Schiavo, *Structure and Function of Tetanus and Botulinum Neurotoxins*, 28(4) Q. Rev. Biophys. 423-472 (1995).

Giampierto Schiavo et al., *Neurotoxins Affecting Neuroexocytosis*, 80(2) Physiol Rev 717-766 (2000).

Michael Veit et al., *Multiple Palmitoylation of Synaptotagmin and the t-SNARE SNAP-25*, 385(1-2) FEBS Lett. 119-123 (1996).

X. RELATED PROCEEDINGS

None.